ACTA MEDICA
SCANDINAVICA

SUPPLEMENTUM 441

SERUMINSULIN

A review

BY

JENS LYNGSØE

ACCOMPANIES VOL. 179

AARHUS 1965

ACTA MEDICA SCANDINAVICA

has been published since 1919 as a continuation of *Nordiskt Medicinskt Arkiv, founded in 1869 by Axel Key*. The first volume of Acta Medica Scandinavica is therefore numbered LII (52).

The chief editors have been Axel Key 1869–1900, C. G. Santesson 1901–1915, I. Holmgren 1916–1957 and Birger Strandell 1958 to date.

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The annual subscription to the journal, covering two volumes, each of 6 numbers, is 140 Sw. crowns or U. S. $ 27.25 *including postage*; in the Scandinavian countries and in Holland 120 Sw. crowns.

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ACTA MEDICA SCANDINAVICA

P. O. Box 2052, Stockholm 2

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SERUMINSULIN

FROM THE SECOND UNIVERSITY CLINIC OF INTERNAL MEDICINE
(Chief Professor K. LUNDBÆK M.D.)
KOMMUNEHOSPITALET, ÅRHUS DENMARK

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Translated from the Danish by H. Cowan, B.Sc.

Authors' Previous Publications on This Subject

1 Determination of the Insulin-like Activity in Serum Using Rat Epididymal Adipose Tissue *Scand J Clin Lab Invest* *13* 628–636 1961

2 The Insulin-like Activity in Serum Determined by the Rat Epididymal Fat Method I Normal Values in Undiluted and Diluted Serum and the Effect of Ingestion of Glucose *Acta Med Scand* *171* 365–375 1962

3 The Insulin-like Activity in Serum Determined by the Rat Epididymal Fat Method II The Values in Undiluted and Diluted Serum from Diabetic Patients Determined before and after the Ingestion of Glucose *Acta Med Scand* *172* 41–62 1962

4 The Insulin-like Activity in Serum Determined by the Rat Epididymal Fat Method III Insulin Antagonism in Serum from Normal Persons and Diabetics Including some Observations on Pig's Blood *Acta Med Scand* *172* 601–614 1962

5 The Insulin-like Activity in Serum Determined by the Rat Epididymal Fat Method IV Anti-insulin Inhibition of Insulin-like Activity in Electrophoretically Separated Serum Protein Fractions *Acta Med Scand* *174* 589–594 1963

6 The Insulin-like Activity in Serum Determined by the Rat Epididymal Fat Method V The Distribution of Insulin-like Activity in Electrophoretically-separated Serum Protein Fractions from Normal Fasting Subjects and the Effect of Ingestion of Glucose *Acta Med Scand* *175* 401–408, 1964

PREFACE

The present work was carried out during my employment at the Second University Clinic of Internal Medicine, Kommunehospitalet, Århus, Denmark. Head Professor Knud Lundbæk, M.D.

The thesis is based on investigations made during the years 1959 to 1963.

To Professor Knud Lundbæk I wish to express my profound gratitude for ideal working conditions and encouragement. Professor Lundbæk's great interest in the subject of this study has contributed much to the completion of this work.

Professor Willy Munck, M.D., former head of the Department of Pathology, Århus Kommunehospital, and Professor T. Steen Olsen, M.D., head of the Department of Pathology, Århus Kommunehospital generously gave me laboratory space and facilities.

My thanks are due to Dr. N. R. Kerding, M.D., head of the Central Laboratory, Århus Kommunehospital, who helped with some special technical problems, and with whom much valuable discussion was shared.

I wish to express my gratitude to Professor C. J. Munch Petersen, M.D., head of the Department of Neurology, Århus Kommunehospital, and to Professor Cai Holten, M.D., former head of the First University Clinic of Internal Medicine, Århus Kommunehospital, who allowed me to examine patients in their departments.

Svend Darling, Ph.D., Professor of Biochemistry, kindly allowed me to use the laboratory facilities of the Department of Biochemistry, Århus Tandlægehøjskole.

I wish to thank my collegue Dr. H. C. Ørskov for reading through part of the manuscript and for many fruitful discussions.

In particular, I am indebted to my laboratory assistant Mrs. Tove Dreyer Sørensen, who indefatigably aided me during the whole period of investigation.

CONTENTS

To the nurses of the Second Department of Internal Medicine I am indebted for close cooperation in the supervision of the patients selected for examination My thanks are extended to Librarian Miss L Thomsen for help in obtaining the relevant literature

The investigations were made possible by grants from Statens almindelige Videnskabsfond, Reinholdt W Jorck og Hustru's fond, Fonden til Lægevidenskabens fremme and Novofonden, which are herewith gratefully acknowledged

Finally, I owe a great debt of gratitude to my wife Karin, who afforded invaluable assistance through her constant encouragement in the course of the preparation of this monograph

Copenhagen, October 1965

Jens Lyngsøe

CONTENTS

INTRODUCTION

The elucidation of the pathogenic factors in diabetes mellitus and other diseases which probably involve abnormalities of insulin production, has been hampered by methodological problems in the determination of the insulin content of the serum. The methods which have been elaborated for this determination are technically very difficult, and different investigators have obtained highly divergent results, even when one and the same method was used. For years, no satisfactory explanation could be found for the lack of agreement between serum insulin values obtained in these investigations. It is only in the last few years, with the publication of studies suggesting that serum contains several different forms of insulin, that there has been hope of a satisfactory theory emerging to explain the divergences mentioned. At the same time, an understanding of the mode of secretion of insulin also appears to be within reach, such an explanation is a prerequisite for elucidating the pathogenesis in diabetes mellitus and in the different hypoglycaemic states.

In the present study, the literature on serum insulin has been covered till the autumn of 1963. Previous summaries of the literature have been made by Willebrands & Groen (1954) (293), Vallance Owen & Wright (1960) (279), and Yalow & Berson (1960) (309).

Definitions and abbreviations

Insulin like activity (ILA) the activity which can be measured by a biological method for determining insulin

Serum insulin like activity (SILA) the activity which can be measured by a biological method in untreated, undiluted or diluted blood, serum and plasma

Immunologically determined insulin the activity which can be measured by an immunological method for determining insulin

Hidden ILA the insulin like activity which, in untreated undiluted or diluted serum, is biologically and/or immunologically inactive

Suppressible SILA that part of SILA (measured by the rat epididymal fat method) which is inhibited after addition of anti insulin

Non suppressible SILA that part of SILA (measured by the rat epididymal fat method) which is not inhibited after addition of anti insulin

Insulin antagonist serum factors which in vitro inhibit the effect of insulin on an isolated tissue

'*A fraction* ILA localized to albumin-α_1 globulin

B fraction ILA localized to β γ globulin

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TABLE 1

In vivo techniques for the determination of small quantities of insulin

Preparation	Administration	Parameter registered	Sensitivity μU/ml	Precision (mean lambda)	
Intact mice	SC	Convulsions	2000–4000	–	Stewart (1960)[158]
Intact mice	IV	Fall in blood sugar	1000	0.52	Beigelman (1958)[12]
Intact rats	IV	do	000	0.15	Arquilla et al (1962)[10]
Intact rats	IP	Glycogen synthesis in the diaphragm	100	0.33	Rafaelsen (1961)[136]
Adrenalectomized mice	SC	Convulsions	2000	–	Hemmingsen & Krogh-Nielsen (1938)[115]
Hypophysectomized rats	IV	Fall in blood sugar	1000	0.22	Anderson & Wherry (1962)[4]
Adreno-demedullated hypophysectomized rats	IV	do	300		Gellhorn et al (1941)[91]
do	IV	do	200	0.16	Kosaka et al (1963)[126]
Alloxan-diabetic hypophysectomized rats	IV	do	2000	–	Randle (1954)[139]
	IV	do	2500	0.31	Beigelman et al (1956)[26]
Alloxan-diabetic hypophysectomized mice	IV	do	1000–2000	–	Goetz et al (1954)[99]
do	IV	do	250	0.70	Anderson et al (1957)[4]
Alloxan diabetic adrenalectomized mice	IV	do	1000		Baird & Bornstein (1959)[17]
Adreno-demedullated alloxan diabetic hypophysectomized rats	IV	do	125		Anderson et al (1947)[1]
Alloxan-diabetic hypophysectomized adrenalectomized rats (ADHA rats)	IV	do	50		Bornstein (1950)[34]

IV intravenous administration
IP intraperitoneal administration
SC subcutaneous administration

cision (lambda) a statistical expression which includes an estimate both of the steepness of the dose effect curve, and of the deviation of the observations around the dose effect curve (87). A low lambda value expresses a high degree of accuracy. It appears from table 1 that lambda for the *in vivo* methods is high, between 0.3 and 0.7. The strikingly low lambda in the study by Ar

IN VIVO METHODS

Brugsch & Horster published the first attempt at measuring the content of insulin in serum in 1930, when they examined an acid alcohol extract of serum by means of the mouse *convulsion test*, normally used for measuring the insulin content of pancreatic extracts It was found, however, that intact mice could not be used for experiments to determine the insulin like effect of this extract (45) Hemmingsen & co-workers (119) showed that by removing the adrenals, the sensitivity of rats and mice to insulin could be increased Gellhorn worked out a more sensitive technique, in which he used the decrease in blood sugar in hypophysectomized, adrenodemedullated rats as an indicator of the insulin activity (91) This technique made it possible to demonstrate insulin-like activity in serum for the first time Since then, other *in vivo* methods have been worked out, all of them based on the same principle the insulin sensitivity of the animals is increased by removal of one or several of the endocrine organs which take part in the regulation of the blood sugar Table 1 is a schematic presentation of these methods, their sensitivity and their accuracy

Rafaelsen (196) introduced an *in vivo* technique which was based on a new principle He measured the changes in glycogen content in the diaphragm of the rat after an intraperitoneal injection of serum or standard insulin solutions into the intact animal 100 μU of insulin per ml solution was found to increase the glycogen content, and an insulin like effect of serum could be determined by this method

All the *in vivo* techniques are very difficult to work with on the whole (183, 203), and few studies using these methods have been published The only methods which have proved sufficiently sensitive to register insulin like activity in serum are those of Gellhorn, Bornstein and Rafaelsen The other methods have been used mainly for determining the insulin like activity in protein concentrates or extracts from serum (2, 17, 25, 99)

Studies using *in vivo* techniques are of considerable interest, particularly when applied to measuring the insulin like activity of serum from the same animal species as the test animals Under such circumstances, these studies will give the most accurate measurement of the physiological insulin like activity of the serum, *i e* corresponding to conditions in which insulin exerts its normal physiological activity

Sensitivity

It appears from table 1 that the sensitivity of the *in vivo* methods varies considerably The Bornstein method by which 50 μU/ml can be determined, is the most sensitive method, while the other methods have sensitivities varying from 100 to 7000 μU/ml

Precision

The accuracy of biological methods is generally quoted as an index of pre

method for determination of glycogen used in their study, however, the effect of insulin on the glycogen synthesis can only be demonstrated with insulin concentrations considerably higher than those which will increase the glucose uptake in rat diaphragm (194 244) Perlmutter & co-workers were thus unable to demonstrate ILA in serum by this technique Recently, however, a method for determining insulin has been published which uses the synthesis of free glycogen in the rat diaphragm as a parameter for the insulin effect This method seems to be sufficiently sensitive to determine SILA (128) Although it appears very promising, the method has not yet been taken up by other investigators

Some studies use Stadie s "dipping technique (246) for the examination of insulin antagonism in serum (see chapter 3) With this technique, the synthesis of glycogen is measured in rat diaphragm which has first been dipped for a short period in a solution of insulin, and then incubated in a buffer without insulin This technique cannot be used for determing SILA but by comparing the glycogen content of two hemi diaphragms, one of which has been dipped in an insulin solution, the other in a mixture of serum and insulin solution it is possible to obtain a measure of the insulin inhibiting effect of serum

A single study has been published in which the incorporation of C^{14} marked amino acids into rat diaphragm protein is used as a measure of the effect of the insulin This method is sufficiently sensitive to demonstrate insulin like activity in serum (164)

The rat diaphragm method has been used in a considerable number of studies on insulin like activity in serum By far the majority of these investigations use the glucose uptake as a measure of the insulin effect With this method, one part of a diaphragm is incubated in serum, and another part of the same diaphragm is incubated in a buffer without insulin The difference between the uptake of glucose in the two incubation vessels, the excess glucose uptake", can then be ascribed to the serum, and is used as a measure of the insulin like activity of the serum On comparing the excess glucose uptake in the serum with the value measured by other diaphragms incubated in buffer containing a known insulin content, it is possibly by interpolation to find an insulin concentration giving an excess glucose uptake equivalent to that of the serum This concentration will then indicate the insulin like activity of the serum

A mouse diaphragm method has been worked out which in principle corresponds in every way to the rat diaphragm method (180, 181, 291)

Table 2 shows the variants of the rat diaphragm method using glucose uptake as a measure of the insulin activity In most of these investigations, the rat diaphragm is washed in iced buffer immediately following removal, as a result, a reduced variation is obtained between the glucose uptakes in diaphragms from different animals (44) It appears from table 2 that most investigators have used

quilla & co-workers (15) *is due to the* fact that in this form of assay, a great *number of* animals are *employed for* measuring the insulin-like activity. A *number of the* studies cited do not give any *information* as to the *precision* of *their methods.*

Specificity

There are only a few studies in which the specificity of the *in vivo* methods is elucidated. Gellhorn & co-workers (92) found no ILA in serum from pancreatectomized dogs. Baird & Bornstein (17) showed with adrenalectomized, alloxan-diabetic mice that the insulin-like activity in an alcohol extract of serum disappears following treatment with cysteine, which is known to inactivate insulin. In studies with this method, the addition of insulin to the extract *did not* show any insulin-inhibiting factors in the extract.

Rafaelsen (197) used his method to examine *the effect* of hormones on the synthesis of glycogen in the rat diaphragm. Growth hormone in amounts of 10 µg/rat, glucagon (1 µg/rat) and desoxycorticosterone (10 µg/rat), increase the deposition of glycogen. Epinephrine (1 µg/rat) decreases the glycogen content of the rat diaphragm. Thus, these hormonal effects are found with amounts of hormone which are considerably greater than the amounts normally present in the volume of serum injected when Rafaelsen's method is used for determining SILA. Nevertheless, the possibility is not excluded *that* even smaller amounts of hormone *may influence* SILA determinations by *this method. It* has not been proved, *therefore, that* those SILA *values* determined by Rafaelsen's method are independent of the hormones which have been studied. The fact that acetyl salicylic acid can increase the glycogen content of the rat diaphragm *in vivo*, may be of significance in the study of SILA in man.

THE RAT DIAPHRAGM METHOD

Gemmill was the first investigator to demonstrate the effect of insulin on glucose uptake and glycogen synthesis in the isolated rat diaphragm (93, 94, 95). These findings were subsequently confirmed by other investigators (136, 217, 244, 265, 285). Studies on the metabolism of the rat diaphragm have suggested that insulin stimulates the conversion of glucose to pyruvate and to carbon dioxide (284, 285) and shown that insulin stimulates the incorporation of amino acid in protein (137, 161, 238).

Groen & co-workers, in 1952, published a method for determining insulin like activity in serum using rat diaphragm as metabolically active tissue (105). These authors used glucose uptake as a measure of the insulin effect and showed that this technique could register the ILA in serum. At the same time Perlmutter & co-workers (186) attempted to measure the insulin like activity in serum by a rat diaphragm technique in which the synthesis of glycogen was used as an index of the insulin effect. With the

hemi-diaphragms, it is stated that further division of the diaphragm will result in a less pronounced *insulin response*, *i e* less difference between the glucose uptake in buffer with and without insulin (279) Some workers have used a 'pooling technique, *i e* portions of diaphragms from several animals are incubated in the same incubation vessel As a result, a correction is obtained for any difference in the glucose uptake which may be present in diaphragms from different animals Bicarbonate buffers were used to incubate the diaphragms in all methods except one, where a *Krebs-Ringer phosphate* buffer was used (259) Only a few workers have added a carrier protein (for instance gelatine) to the standard insulin solutions (63 236) Such an addition will prevent the insulin in the solution from being adsorbed to the walls of the incubation vessels, whereby it is hindered in its effect on the diaphragm (121) Cunningham (63) could thus demonstrate that gelatine had a distinct influence on the effect of insulin on the glucose uptake of the diaphragm

Comparing the glucose uptake in diaphragm samples incubated under similar test conditions the glucose uptake is *considered in relation to the size of the* piece of diaphragm In most studies, the glucose uptake is calculated in relation to the weight of the diaphragm piece Previously it was *considered important* for the accuracy of the experiments whether the glucose uptake had been calculated per g wet weight or per g dry weight As the water *content* of the rat diaphragm is fairly constant, however (161, 194, 279), this point *is of no* significance The water content is known to be 75 % of the total weight, so that a glucose uptake per g dry weight can be converted to a glucose uptake per g *wet weight* (Table 2)

In one investigation in which rats of almost identical weight were used, no correction was made for the diaphragm weight, the glucose uptake being calculated per hemidiaphragm (295)

As was anticipated, it appears from table 2 that the basal glucose uptake of the rat diaphragm, *i e* the uptake in a *buffer without insulin*, varies *considerably in* different studies The excess glucose uptake resulting from a definite amount of insulin (in the table 100 μU/ml) also varies considerably, *viz* from 0 6 to 3 5 mg glucose/g diaphragm/90 minutes incubation time This value varies in different rat strains and in animals of different size. A low excess glucose uptake, therefore, need not necessarily signify an unfortunate combination of the technical details mentioned above It appears from table 2, however, that with a single exception, *the* sensitivity to insulin in the rat diaphragm method seems to be proportional to the excess glucose uptake produced by 100 μU insulin per ml buffer

Piazza & co-workers (192) showed that when the rat diaphragm method is used for measuring SILA, the amount of incubated tissue per *incubation* vessel is significant Using I^{131} labelled insulin, these authors compared the insulin break-down in standard insulin solutions,

TABLE 2

Diaphragm methods

	Division of the diaphragm¹)	Pieces of diaphragm per incubation vessel	Cooling²)	Time of incubation min.	Glucose-concentration mg %	Basal glucose-uptake mg/g/90 min.³)	Excess glucose-uptake 100 µU per ml mg/g/90 min	Sensitivity µU/ml
Rat diaphragm								
Randle (1954)[199,200] (1956)[202]	1/2	6	–	180	250	7.0 (per 180 min.)	1.9 (per 180 min.)	100–2000
Groen et al. (1952)[106]	1/2	4	+	90	200	7.6	2.0	10
Willebrands & Groen (1956)[294]	1/8	8	+	90	200		0.8	100
Tacheuchi et al. (1957)[259]	1/2	4	+	90	200		1.1	1–10
Vallance Owen et al. (1954)[271], Wright (1957)[302], Seltzer & Smith (1959)[235]	1/2	1	+	90	300	7.8	3.4	10
Staub (1958)[248]	1/2	1	+	120	200	4.5 (per 120 min.)	0.6 (per 120 min.)	100
Willebrands et al. (1958)[295]	1/2	1	+	90	150	0.23 (per hemidiaphragm)	0.15 (per hemidiaphragm)	30
Metz (1960)[170]	1/2	1	+	60	20–700		2.6	
Cunningham (1962)[62]	1/2	1	–	90	250	3.6	3.4	10
Jessup & Wiberg (1961)[128]	1/8	1	+	90	200	5.7	2.7	25
Mouse diaphragm								
Oyama & Grant (1960)[181]	1/2	4	–	90	100	9.5	1.0	100
Wiberg & Jessup (1961)[291]	1/2	5	+	90	100	7.0	1.4 (produced by 50 µU/ml)	10

¹) 1/2 indicates that hemidiaphragms have been used.
1/5 indicates that the hemidiaphragms have been cut into 5 pieces.
1/8 indicates that the hemidiaphragms have been cut into 8 pieces.
²) Indicates whether the diaphragms have been washed in a cooled buffer before the incubation.
³) Calculated per g wet weight (see pg. 19)

(see page 19) Where no carrier protein is present in these solutions, it may be assumed that part of the insulin will be adsorbed to the glassware As a result, the excess glucose uptake measured in standard insulin solutions, will correspond to a lower insulin concentration than was intended In agreement with this argument, it is found that the excess glucose uptake provoked by 100 µU insulin per ml buffer, is higher in the variant of the rat diaphragm method in which gelatine is used in the standard insulin solutions (63), than it is in the great majority of the other variants (see table 2)

Sensitivity

Table 2 shows that the rat diaphragm methods which use glucose uptake as a measure of the insulin effect, vary considerably in sensitivity A number of investigators report being able to demonstrate 10 µU insulin per ml incubation medium whereas others only find an effect with insulin concentrations of 100 µU/ml or more More or less the same sensitivity seems to be obtained by the mouse diaphragm method as insulin concentrations from 10 to 100 µU/ml can be demonstrated

It is reported that 25 µU/ml can be demonstrated by the rat diaphragm method, which uses glycogen deposition as a measure of the insulin effect (128), whereas 50 µU/ml can be determined by the method of C^{14} glycine incorporation in diaphragm protein (164)

Precision

Studies in which the precision of the rat diaphragm method is calculated, indicate this value by the index of precision lambda Randle (202), in his modification of the method, found an average lambda value of 0 34 Vallance-Owen (269) states that his modification shows lambda values from 0 18 to 0 28, while other workers using the same modification find lambda values from 0 20 to 0 55 (mean 0 28) (18) Takeuchi (259), using his own method, finds lambda values of the same magnitude (mean 0 28), while the method which uses rat diaphragm divided into 8 pieces, shows a higher lambda value (mean 0 51) (128)

Lambda values between 0 09 and 0 38 (mean 0 24) are found by the mouse diaphragm method (291) The same authors report that the rat diaphragm method, in which glycogen deposition is used as a measure of the insulin activity, has a lambda value of 0 30 (128)

Specificity

A range of studies suggest that the rat diaphragm method measures an effect of insulin in serum Most of the studies elucidating this problem have employed a modification of the method which measures SILA by means of glucose uptake in the diaphragm With this technique, it has been demonstrated that SILA disappears following treatment of the serum with cysteine or glutathione, which are known to inactivate insulin

in three variants of the rat diaphragm method They found that the insulin break-down in standard insulin solutions was greatest with Randle's method, less with Groen's method and least with Vallance-Owen's method This is in agreement with the finding that the insulin break-down is proportional to the number of incubated hemidiaphragms per ml incubation fluid

On the basis of Piazza's investigations, therefore, it might be anticipated that in all modifications of the rat diaphragm method, but most pronounced in the variants with "pooling" techniques, the standard insulin solutions would give an excess glucose uptake which corresponded to a lower insulin concentration than intended With one exception, the results obtained are in fact in accordance with those of Piazza The excess glucose uptake elicited by 100 μU insulin per ml buffer was found lowest in those variants of the rat diaphragm method in which several hemidiaphragms were used per incubation tube (see table 2)

Using the rat diaphragm method to determine the serum insulin-like activity, the excess glucose uptake due to the serum is related to the uptake elicited by insulin In studies on insulin concentration and excess glucose uptake, different relationships were found between dose and response In some studies a linear dependence was found between the cube root of the excess glucose uptake and the logarithm of the insulin concentration in the range 100–10 000 μU/ml (199, 202, 294), other investigators found a linear dependence between the excess glucose uptake and the square root of the insulin concentration in the range 10–1000 μU/ml (272, 302) The majority of investigators found a linear dependence between the glucose uptake and the logaritm of the insulin concentration from 10–1000 μU/ml (170, 235, 248, 291, 295, 259) Cunningham (63) found a linear dependence between the glucose uptake and the insulin concentration from 10 to 150 μU/ml

A linear dependence between the excess glucose uptake and the logarithm of the insulin concentration was found in the few studies in which the mouse diaphragm method was used (181, 291) Wardlaw & Moloney (287) described a mouse diaphragm method in which the insulin effect was measured by glycogen deposition in the diaphragm Using this technique, and adding anti insulin to the incubation vessel, an increase in the accuracy of the method was obtained at the expense of the sensitivity These investigators found that over the range 10,000 μU/ml to 20,000 μU/ml there was a linear dependence between the glycogen synthesis, expressed as a percentage of the glycogen content of the diaphragm incubated in buffer without insulin, and the insulin concentration

In using the rat diaphragm method to determine the serum insulin like activity it is impossible to state with certainty which technical details are important and which are unimportant It is reasonable, however, to attach considerable importance to the presence of a carrier protein in the standard insulin solutions

(see page 19) Where no carrier protein is present in these solutions, it may be assumed that part of the insulin will be adsorbed to the glassware As a result, the excess glucose uptake measured in standard insulin solutions, will correspond to a lower insulin concentration than was intended In agreement with this argument, it is found that the excess glucose uptake provoked by 100 μU insulin per ml buffer, is higher in the variant of the rat diaphragm method in which gelatine is used in the standard insulin solutions (63), than it is in the great majority of the other variants (see table 2)

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(105, 199, 271) Other investigators have demonstrated that serum to which anti-insulin has been added neither stimulates glucose uptake nor effects the incorporation of C^{14}-glycine into diaphragm protein (164, 274, 303) In dogs, Metz (170) found a higher SILA in blood from the pancreatico-duodenal vein than in blood from the femoral artery SILA cannot be demonstrated by the rat diaphragm method in pancreatectomized cats (275), and in dogs which have been pancreatectomized, a steady fall i SILA sets in following the pancreatectomy, decreasing gradually for 20 days until less than 10 % of the initial value (105)

These investigations seem to indicate that when measured by the rat diaphragm method, SILA is due to insulin It is an open question, however, whether the measurement of SILA by this technique indicates solely the amount of biologically active insulin in serum, or whether serum contains factors of perhaps hormonal character, which influence the SILA determinations by inhibiting or stimulating the activity of insulin Table 3 lists investigations of the *in vitro* effect of different hormones on *glucose uptake and glycogen synthesis* in isolated rat diaphragm from normal animals The table shows that in a few studies, growth hormone was found to have an insulin-like effect on this tissue, but as this effect can only be demonstrated in a phosphate buffer and not in a bicarbonate buffer (207), growth hormone is unlikely to influence the determination of SILA by the rat diaphragm method Table 3 shows that a variety of hormones inhibit both the glucose uptake and the glycogen synthesis in rat diaphragm The inhibiting effect of epinephrine on these metabolic processes is found at epinephrine concentrations in the incubation medium which correspond to the concentrations normally found in serum Norepinephrine in physiological concentrations likewise inhibits glucose uptake in rat diaphragm On the other hand, the inhibiting effect of cortisol, corticosterone, desoxycorticosterone and glucagon on the metabolic processes in rat diaphragm is demonstrated at hormone concentrations which are considerably higher than physiological levels The stimulation of C^{14}-glycine incorporation by ACTH has likewise been demonstrated at concentrations far higher than the concentration in serum (163) These investigations, however, do not exclude the possibility that the hormones mentioned may have an effect on the metabolic processes in rat diaphragm even in physiological concentrations

The cited studies of the effect of norepinephrine and epinephrine on the glucose metabolism of the rat diaphragm *suggest that the presence of these hormones in serum can inhibit the effect of insulin, and that measured by the rat diaphragm method therefore SILA can hardly be a quantitative indication of the amount of biologically active insulin in serum* As it is possible, however to recover quantitatively the amount of insulin which has been added to undiluted serum (170, 199 271), it must be sup-

TABLE 3

The in vitro effect of hormones on the glucose metabolism in rat diaphragm

	The hormone concentration in the serum of normal subjects (mean value or range)		Glucose uptake		Glucogen synthesis
Growth hormone	200 (80–300) mμg/ml Ehrlich & Randle (1961)[75]	+	10 μg/ml Ottaway (1953)[177]	0	500 μg/ml Stadie et al. (1949)[246]
	50–400 mμg/ml Read & Bryan (1960)[218]	+	1 μg/ml Ottaway (1951)[178]		
	170 (90–250) mμg/ml Hartog & Fraser (1961)[117]	+	25 μg/ml Randle & Whitney (1957)[203]		
	5 μg/ml McGarry et al (1960)[166]	0	100 μg/ml Randle & Young (1956)[204]		
	0–50 mμg/ml Unger et al (1962)[267]	0	10–100 μg/ml Park et al (1952)[193]		
		0	50 μg/ml Manchester & Young (1959)[162]		
ACTH	0.1 mU/100 ml Fujita (1957)[86]	0	200 μg/ml Ottaway & Bulbrook (1955)[179]		
	1.0 mU/100 ml Bethune et al (1958)[32]	0	25 μg/ml Randle & Young (1957)[205]		
	200 mU/100 ml Bornstein & Trewhella (1950)[38]				
	160 mU/100 ml Montanari et al (1951)[172]				
	35 mU/100 ml Martinelli & Montanari (1955)[164]				
	110–170 mU/100 ml Ceresa & Reyneri (1953)[44]				
Cortisol	5–25 μg/100 ml Eik-Ness (1960)[76]			–	50 μg/ml Stadie et al (1951)[246]
Corticosterone	2–6 μg/100 ml Eik-Ness (1960)[76]	0	10 μg/ml Bartlett & Wick (1949)[21]	–	10 μg/ml Bartlett & Wick (1949)[21]
Desoxycorticosterone		0	10 μg/ml Bartlett & Wick (1949)[21]	–	10 μg/ml Bartlett & Wick (1949)[21]
		–	40 μg/ml Leupin & Verzar (1949)[147]	–	40 μg/ml Leupin & Verzar (1949)[147]

TABLE 3 (continued)

	The hormone concentration in the serum of normal subjects (mean value or range)	Glucose uptake	Glycogen synthesis
Epinephrine	0.6 µg/100 ml Cohen & Goldenberg (1957)[59] 0.0 µg/100 ml Valk & Price (1956)[258] 2.5 µg/100 ml Weil Malherbe & Bone (1954)[88] 11 µg/100 ml Weil Malherbe & Bone (1957)[280] 2.4 µg/100 ml Griswold (1958)[101]	– 0.20 µg/ml Walaas & Walaas (1956)[284] – 0.01 µg/100 ml Groen et al (1958)[106]	– 0.25 µg/ml Walaas & Walaas (1956)[284] – 0.33 µg/ml Tuerkischer & Wertheimer (1948)[263] – 5 µg/ml Riesser (1947)[217]
Norepinephrine	3 µg/100 ml Cohen & Goldenberg (1957)[59] 2 µg/100 ml Valk & Price (1956)[258] 41 µg/100 ml Weil Malherbe & Bone (1957)[280] 57 µg/100 ml Weil Malherbe & Bone (1954)[288] 32 µg/100 ml Griswold (1958)[101]	– 0.75 µg/ml Walaas & Walaas (1956)[284] – 0.05 µg/ml Groen et al (1958)[106]	1.0 µg/ml Walaas & Walaas (1956)[284]
Glucagon	350 (0–900) µµg/ml Unger et al (1962)[264]	50 µg/ml Candela (1953)[51]	100 µg/ml Snedecor et al (19[illegible])[illegible]

\+ indicates that a stimulation has been registered
– indicates that an inhibition has been registered
0 indicates that no effect has been registered

posed that the presumed epinephrine norepinephrine inhibition in undiluted serum has been compensated by the insulin present

Several investigations suggest that other substances than insulin are able to increase the glucose uptake and the glycogen synthesis in rat diaphragm. Castrillon & co-workers (55) have thus briefly reported that in a concentration corresponding to the physiological level (2 mg% (250)) leucine will increase both these metabolic processes. Haraldsen & Walaas (125), in a detailed study found an ultrafiltrable serum factor which stimulated the glucose uptake in

rat diaphragm The glucose uptake is likewise increased by carbutamide and tolbutamide (195), phenethyldiguanide 57, 195, 296), sodium salicylate (162) and a number of enzyme poisons such as 2 4 dinitrophenol, sodium arsenate and sodium cyanide (208)

THE RAT EPIDIDYMAL FAT METHOD

Rat epididymal fat was used for the first time in metabolic studies in 1954 (118) Later investigations showed that insulin increased epididymal fat s glucose uptake and oxidation of glucose to carbon dioxide *in vitro* (126 297) These findings have later been confirmed in numerous studies (53 62 69 111 112, 141 146 152 305) It has further been demonstrated that insulin stimulates the rat epididymal fat s conversion of glucose to lactate (112) to glycogen (49 146) and to fatty acids (49, 146, 298)

Renold & co-workers (166) worked out the method for determining the insulin like activity of serum by using rat epididymal fat as a metabolically active tissue At the same time v der Geld Willebrands & Groen (90) published a similar technique with rat mesenteric fat This latter technique has not been rechecked whereas Renold s rat epididymal fat method has been used in a great number of investigations In the study cited Renold used the oxidation of glucose 1 C^{14} to $C^{14}O$ as a measure of the insulin effect but it is possible to use all the above mentioned insulin susceptible metabolic parameters to determine the insulin like activity in serum All the major studies so far published on SILA use the fatty tissue's glucose uptake, oxidation of glucose 1 C^{14} to $C^{14}O_2$, and carbon dioxide production, to measure SILA

The method whereby SILA is measured by the carbon dioxide production, utilizes the fact that insulin provokes an increase in the respiratory quotient of fatty tissue, and of the organism as a whole This increase is due to an increase in the carbon dioxide production, whereas the oxygen uptake only shows small and uncertain variations It has therefore been found permissible to measure the resulting increase in pressure, the net gas exchange', in a Warburg apparatus as expressing the increase in the respiratory quotient

In the modifications of the rat epididymal fat method mentioned, advantage is taken of the fact that it is possible to establish a linear dependence between the insulin concentration in the incubation medium and the metabolic response of the fatty tissue to insulin In calculating this relation, allowance must be made for the fact that the weight of incubated fatty tissue is not the same in all incubation vessels A large fat sample will have a greater glucose metabolism than a small fat sample, even under otherwise constant conditions Renold (166) made a correction for this by calculating the amount of $C^{14}O_2$ developed per g fatty tissue Other investigators, using smaller fat samples, found that the ratio

$$\frac{\text{amount of } C^{14}O_2 \text{ developed}}{\sqrt{\text{fat weight}}}$$

TABLE 3 (continued)

	The hormone concentration in the serum of normal subjects (mean value or range)	Glucose uptake	Glycogen synthesis
Epinephrine	0 6 µg/100 ml Cohen & Goldenberg (1957)[50] 0 0 µg/100 ml Valk & Price (1956)[268] 25 µg/100 ml Weil Malherbe & Bone (1951)[28] 11 µg/100 ml Weil Malherbe & Bone (1957)[289] 2 4 µg/100 ml Griswold (1958)[101]	− 0 20 µg/ml Walåes & Walåes (1956)[24] − 0 01 µg/100 ml Groen et al (1958)[106]	− 0 25 µg/ml Walåes & Walåes (1956)[286] − 0 33 µg/ml Tuerkischer & Wertheimer (1948)[263] − 5 µg/ml Riesser (1947)[217]
Norepinephrine	3 µg/100 ml Cohen & Goldenberg (1957)[50] 2 µg/100 ml Valk & Price (1956)[268] 41 µg/100 ml Weil Malherbe & Bone (1957)[289] 57 µg/100 ml Weil Malherbe & Bone (1954)[288] 32 µg/100 ml Griswold (1958)[101]	− 0 75 µg/ml Walåes & Walåes (1956)[286] − 0 05 µg/ml Groen et al (1958)[106]	− 1 0 µg/ml Walåes & Walåes (1956)[286]
Glucagon	350 (0–900) µµg/ml Unger et al (1962)[266]	− 50 µg/ml Candela (1953)[41]	− 100 µg/ml Snedecor et al (1955)[243]

+ indicates that a stimulation has been registered
− indicates that an inhibition has been registered
0 indicates that no effect has been registered

posed that the presumed epinephrine-norepinephrine inhibition in undiluted serum has been compensated by the insulin present

Several investigations suggest that other substances than insulin are able to increase the glucose uptake and the glycogen synthesis in rat diaphragm Castrillon & co-workers (55) have thus briefly reported that in a concentration corresponding to the physiological level (2 mg% (250)) leucine will increase both these metabolic processes Haraldsen & Walaas (125) in a detailed study found an ultrafiltrable serum factor which stimulated the glucose uptake in

been found in some minor test series, i e lower lambda values Steelman (249), for example, found constant values less than 0 15, and in other studies the mean values for lambda are given as 0 18 (290) and 0 12 (47) Using the 'net gas exchange method, Burgi & co-workers found the same lambda values The greater accuracy of the latter over the former studies is hardly due to a different metabolic parameter being used for measuring the insulin effect. In the more accurate investigations, however, it is decisive that a greater number of rats were used per assay than in the other studies

Specificity

It has often been discussed whether the rat epididymal fat method, in addition to insulin measures other factors in serum which have the same effect on the glucose metabolism of the fatty tissue as insulin (30 145) This is a conclusion which suggests itself for several reasons For one thing, it has been established that SILA measured by the rat epididymal fat method does not disappear following pancreatectomy whereas insulin measured immunologically, and SILA measured by the rat diaphragm method, both disappear For another thing, SILA *measured by the rat epididymal fat* method is only partially inhibited after addition of anti insulin this also in contrast to experience with the rat diaphragm method In addition several hormones and amino acids such as leucine and glutathione are present in serum, and with respect to some parameters, their effect on the glucose metabolism of the rat epididymal fat corresponds to that of insulin

Using the rat epididymal fat method, a number of investigators (74, 145, 146) have been able to demonstrate SILA in apparently totally pancreatectomized experimental animals, in Goldberg & Egdahl s investigation, in spite of the fact that insulin could not be demonstrated in serum by the immunological method These findings have been adduced as an *argument that in addition to insulin, the* rat epididymal fat method measures other factors in serum The argument, however, is hardly valid Using the rat epididymal fat method, it was thus shown that SILA disappeared in pancreatectomized animals following treatment of serum with gluthatione (257) The same investigators also found that 5 days after pancreatectomy in dogs, extraction of the serum with acid alcohol gave an insulin like activity in the extract, and part of this activity could be inhibited by anti insulin This investigation would appear to demonstrate unambiguously that insulin is found in serum some time after ' total pancreatectomy The reason for this has not been elucidated, but investigations by Samaan & co-workers (229) are of considerable interest in this connection These workers found that apparently totally pancreatectomized dogs presented a pronounced hypoglycaemic reaction following injection of alloxane. They claimed that this phenomenon supported the theory that ectopic pancreatic tissue,

gives a better correction for variations in the fat weights (152, 240) In agreement with this, both Renold (216) and Beigelman (24) found that the glucose metabolism per g of fatty tissue is *higher* for fat pieces less than 70 mg than it is for larger pieces

In *their first studies*, Renold & coworkers found a linear relationship between the logarithm of the insulin concentration in the range 30 to 500 μU/ml *and the square root* of the amount of $C^{14}O_2$ developed/g fatty tissue (166) In a subsequent study, however, they showed that by using the relation between *the logarithm* of the amount of $C^{14}O_2$ developed/g fatty tissue and the logarithm of the insulin concentration, there was less variation in the SILA determinations (lower lambda) (216) This linear dependence between log dose and log response was confirmed by later investigators (146, 152, 189, 226, 290)

Several investigators used the glucose uptake of the epididymal fat as a measure of the insulin effect, and found a linear dependence between the glucose uptake per g fatty tissue and the logarithm of the insulin concentration (123, 198, 248, 249, 290) Others, again, found a linear dependence between the logarithm of the glucose uptake per g tissue and the logarithm of the insulin concentration (24, 146)

In some studies, the "net gas exchange" of the epididymal fat is used as a measure of the insulin effect Ramseir et al (198) found a linear dependence between the gas evolved per g fat tissue per hour and the logarithm of the insulin concentration in the range 10–1000 μU/ml Another variant of this method calculates the relation between the evolution of gas resulting from incubation of a sample of fat with a solution containing unknown ILA and the evolution of gas in the same incubation vessel, when a very large amount of insulin (0 1 U/ml) is added to the solution, after this has been incubated for some time Between this relation, the "percentage of maximal response", and the insulin concentration, there is a linear dependence in the range 10–100 μU insulin pr ml (19, 140)

Sensitivity

The three modifications of the rat epididymal fat method the use of glucose uptake, the "net gas exchange", and the oxidation of glucose-1 C^{14}, all seem to have more or less the same sensitivity They are all able to measure insulin amounts of 10 μU per ml incubation liquid (23, 111 167, 240, 305)

Precision

In many determinations using the oxidation of glucose-1-C^{14} as a measure of the insulin effect, Renold's group find a mean lambda of 0 30 (237) Other investigators, using the same procedure find mean lambda values of 0 28 (70 152) and 0 22 (290)

Where the glucose uptake of the rat epididymal fat is used as a measure of the insulin effect a greater accuracy has

TABLE 4

The in vitro effects of hormones on the glucose metabolism in rat epididymal fat

	Hormone concentration in serum (from normal subjects)	Glucose uptake	Net gas exchange	Glucose-1-C to CO
Growth hormone	0 mμg 5 μg ml	+ 100 μg/ml Lebeuf & Cahill (1961)[illegible]	0 1 mg/ml Jungas & Ball (1960)[illegible]	+ 200 μg ml Winegrad et al (1959)[illegible]
		+ 1 mg ml Jungas & Ball (1960)[illegible]		+ 1 mg ml Lebeuf & Cahill (1961)[illegible]
		0 100 μg ml Deutschuner et al (1961)[illegible]		0 100 μg ml Deutschuner et al. (1961)[illegible]
ACTH	0 1 200 mU/ 100 ml	+ 100 μg/ml Lebeuf & Cahill (1961)[illegible]		+ 20 μg/ml Lynn et al (1960)[illegible]
				0 100 μg ml Lebeuf & Cahill (1961)[illegible]
Cortisol	5–25 μg/ 100 ml	+ 0 1 μg/ml Deutschuner et al. (1961)[illegible]		0 30 μg/ml Jeanrenaud & Renold (1960)[illegible]
		0 30 μg ml Lebeuf et al (1962)[illegible]		0 10 μg ml Deutschuner et al (1961)[illegible]
Epinephrine	0 25 μg 100 ml	+ 18 μg ml Lebeuf et al. (1959 1961)[illegible]	+ 10 μg ml Hagen & Ball (1960)[illegible]	+ 20 μg/ml Lynn et al. (1960)[illegible]
		10 μg ml Hagen & Ball (1960)[illegible]	+ 1 μg ml Correa & Magelhaes (1961)[illegible]	+ 18 μg ml Cahill et al (1960)[illegible]
		0 0 01 μg ml Humbel (1959)[illegible]		
Glucagon	0 300 (mean 350) μμg ml	+ 0 02 μg ml Deutschuner et al. (1961)[illegible]		0 1 μg/ml Deutschuner et al (1961)[illegible]
		+ 0 01 μg ml Hagen (1961)[illegible]		+ 0 4 μg ml Worner & Weinges (1961)[illegible]
		+ 100 μg ml Lee et al (1960)[illegible]		
		+ 40 μg/ml Worner & Weinges (1961)[illegible]		

For references see table 3

which had not been removed by the operation, must be present in the dogs The reason why the insulin in serum from pancreatectomized dogs cannot be demonstrated by the immunological method or by the rat diaphragm method, is presumably either that the insulin is present in a state in which it has no effect on the two test systems, or that with respect to the rat diaphragm method, its effect is inhibited by insulin antagonists (275)

In several studies on the rat epididymal fat method, SILA was examined after the addition of anti insulin to serum Between 5 and 30 % of the SILA was found to be inhibited by anti-insulin (47, 146, 159, 198, 226, 227) This was taken as a proof of the hypothesis that in addition to insulin, serum contains other factors with insulin-like activity on rat epididymal fat It has been shown, however, that electrophoretically-separated serum protein fractions contain significant amounts of insulin in an immunologically inactive state (156) It is probable that the SILA fraction which is not inhibited by anti insulin in the investigations with the rat epididymal fat method, is due to this immunologically inactive insulin

A number of investigations support the assumption that the insulin-like activity which can be registered by the rat epididymal fat method, is due to insulin For example, it has been demonstrated that SILA measured by the rat epididymal fat method disappears following treatment of serum with cysteine or glutathione, which inactivate insulin (216 226) It has been shown that SILA is higher in pancreatic venous blood than in peripheral venous blood (167, 187, 226), and that there is a rise in SILA following glucose administration in normal subjects (48, 187, 153, 190, 226, 227) The addition of insulin to undiluted serum is followed by a rise in SILA, corresponding to the amount of insulin added (96, 123, 155, 187)

In order to further characterize the ILA in serum, two investigators have examined the simultaneous action of serum on a number of metabolic processes in the fat Ramseir et al (198) examined the effect of serum on the following processes in rat epididymal fat glucose uptake, net gas exchange, oxidation of uniformly marked glucose C^{14} to $C^{14}O$ and incorporation of C^{14} in fatty acids With these parameters, identical SILA values were found on studying serum diluted five fold Leonards et al (146) examined SILA in a corresponding manner by means of the following parameters glucose uptake, conversion of glucose-1-C^{14} to $C^{14}O$ glycogen, fatty acids and glycerol This investigation showed identical values for SILA with five of the parameters mentioned only the method with incorporation in glycogen resulted in lower values than the other tests Leonards took this result to support the assumption that glycogen synthesis from glucose is a more specific measure of the insulin activity than the other metabolic parameters examined Renold (213), who made extensive determinations of C^{14} incorporation in rat epididymal fat glycogen has suggested

TABLE 4

The in vitro effects of hormones on the glucose metabolism in rat epididymal fat

Hormone	Hormone concentration in serum (from normal subjects)	Glucose uptake			Net gas exchange			Glucose-1-C → CO		
Growth hormone	0 mμg 5 μg ml	+	100 μg/ml	Lebeuf & Cahill (1961)[142]	0	1 mg/ml	Jungas & Ball (1960)[139]	+	200 μg ml	Winegrad et al. (1959)[209]
		+	1 mg ml	Jungas & Ball (1960)[139]				+	1 mg/ml	Lebeuf & Cahill (1961)[142]
		0	100 μg/ml	Ditschuneit et al (1961)[71]				0	100 μg ml	Ditschuneit et al (1961)[71]
ACTH	0.1 200 mU/ 100 ml	+	100 μg/ml	Lebeuf & Cahill (1961)[142]				+	20 μg/ml	Lynn et al (1960)[151]
								0	100 μg/ml	Lebeuf & Cahill (1961)[142]
Cortisol	5 25 μg/ 100 ml	+	0.1 μg/ml	Ditschuneit et al (1961)[1]				0	30 μg/ml	Jeanrenaud & Renold (1960)[127]
		0	30 μg/ml	Lebeuf et al (1962)[143]				0	1.0 μg/ml	Ditschuneit et al (1961)[71]
Epinephrine	0–25 μg/ 100 ml	+	18 μg/ml	Lebeuf et al. (1959 1961)[141, 143]	+	10 μg/ml	Hagen & Ball (1960)[113]	+	20 μg/ml	Lynn et al (1960)[151]
		+	10 μg ml	Hagen & Ball (1960)[113]	+	1 μg ml	Correa & Magelhaes (1961)[4]	+	18 μg/ml	Cahill et al (1960)[40]
		0	0.01 μg/ml	Humbel (1959)[123]						
Glucagon	0–900 (mean 350) μμg/ml	+	0.02 μg/ml	Ditschuneit et al. (1961)[71]				0	1 μg/ml	Ditschuneit et al (1961)[1]
		+	0.01 μg ml	Hagen (1961)[116]				+	0.4 μg ml	Wormer & Weinges (1961)[205]
		+	100 μg/ml	Lee et al (1960)[144]						
		+	40 μg ml	Wormer & Weinges (1961)[205]						

[1]) For references see table 3

that the lower SILA values which Leonards found by means of this technique, might be due to technical problems in connection with the extraction of the very small amounts of glycogen present in fatty tissue, and with the determination of the C^{14} content of the extract

A number of organic compounds have been shown to possess an insulin like effect on the glucose metabolism in rat epididymal fat Table 4 is a schematic representation of those studies made on hormonal *in vitro* effects on metabolic processes in rat epididymal fat employed in methods for determining the insulin-like activity in serum It appears from the table that the glucose uptake in rat epididymal fat is stimulated by growth hormone, ACTH, epinephrine and glucagon in concentrations which are markedly higher than the physiological levels The "net gas exchange" of fatty tissue is stimulated by epinephrine in concentration which are higher than the physiological levels The oxidation of glucose-1-C^{14} to $C^{14}O_2$ is stimulated by growth hormone and epinephrine, possibly also by ACTH and glucagon, but studies on these hormones give contradictory results A single study demonstrated that all metabolic parameters were stimulated by prolactin in concentrations of 50–100 mg% (299) Thus, even though these hormonal effects on the glucose metabolism of the rat epididymal fat have all been demonstrated at hormonal concentrations significantly higher than the physiological levels, this of course does not exclude the hormones having an insulin like effect also in physiological concentrations It is seen from table 4, however, that only few studies have been made on the *effect of physiological concentrations* of hormone on the glucose metabolism of rat epididymal fat

Castrillon & co-workers (55) have reported that leucine in a concentration of 2 mg%, corresponding to the physiological level of leucine in plasma, is able to increase glucose uptake and glycogen synthesis by rat epididymal fat. The study cited does not permit a more exact evaluation of the technique used in particular, it is not possible to decide whether allowance has been made for the fact that fatty tissue from different sites in the lump of epididymal fat, varies in glucose uptake

In a study of the effect of leucine (2 mg%) on the oxidation of glucose-1-C^{14} to $C^{14}O_2$, and in which attention was paid to the above factor, no effect of leucine on this process could be found (158)

When present in high concentrations a number of organic substances occurring in the living organism have an insulin like effect on the oxidation of glucose-1 C^{14} to $C^{14}O_2$ in rat epididymal fat For example, reduced glutathione in a concentration of 30 mM (216) ribonucleic acid adenylic acid and adenosine in a concentration of 100 mg% (73) and oxytocin (100 mU ml) (171) It is of considerable theoretical interest that also the phenylalanyl chain of insulin is able to accelerate the glucose metabolism in fatty tissue (139

The effect of peroral anti diabetic

drugs on rat epididymal fat was examined in a few studies. Tolbutamide and chlorpropamide stimulate the oxidation of glucose 1 C^{14} to $C^{14}O_2$ (215). It is stated that tolbutamide stimulates both glucose uptake and glycogen synthesis in rat epididymal fat (54), but this was not confirmed elsewhere (70). In the latter study, on the other hand, it was found that phenyl ethyl biguanide increased glucose uptake in rat epididymal fat.

IMMUNOLOGICAL METHODS

Arquilla & Stavitsky (13) were the first to employ immunological principles for the determination of small amounts of insulin. They used a haemagglutination-inhibition technique with insulin antibody from immunized rabbits. This method was not sufficiently sensitive to demonstrate insulin in serum. It has been used during recent years, however, for immunological determination of insulin in serum extracts (184, 222).

Yalow & Berson (307) have worked out an immunological method for determining insulin based on a completely new principle. They had found that dissolved insulin labelled with I^{131} is bound to insulin antibody. In solutions with different concentrations of non radioactive insulin and a fixed concentration of I^{131} labelled insulin the amount of antibody bound I^{131} labelled insulin will depend on the amount of non radioactive insulin in the solution. A high ratio between radioactive and non radioactive insulin will result in a large amount of I^{131} labelled insulin being bound to antibody, and a low ratio, a low amount. After separation of free and antibody-bound I^{131}-labelled insulin, the amount of antibody bound I^{131} labelled insulin can be measured immediately by means of its radioactivity. Berson & Yalow found that this separation was possible by means of paper electrophoresis. They showed that in paper electrophoresis, when serum without insulin antibody is added to I^{131}-labelled insulin, the labelled insulin is adsorbed at the site of application (29). In electrophoresis of a mixture of I^{131} labelled insulin and insulin antibody, the bound insulin migrates with the antibody. If electrophoresis is performed on a solution containing a mixture of I^{131} labelled insulin bound to anti insulin and free I^{131}-labelled insulin, two maxima will be found for the radioactivity, one at the site of application, corresponding to the free I^{131} labelled insulin, and one in the γ globulin range, corresponding to the antibody bound insulin. The ratio between the radioactive radiation in these two regions corresponds to the ratio between free and antibody bound I^{131}-labelled insulin. To measure the insulin content of a solution, the solution is mixed with I^{131} labelled insulin, anti insulin is added, and electrophoresis is then carried out on the solution, the ratio between free and antibody bound I^{131}-labelled insulin being measured. On comparison with the ratio obtained between free and antibody bound I^{131}-labelled insulin when using standard insulin solutions, a measure will be ob-

tained for the content of immunologically active insulin in the "*unknown* solution"

Yalow & Berson's method is so sensitive that it can demonstrate insulin in serum It has been used by other *investigators*, who were able to reproduce Berson & Yalow's results (230)

After the publication of Yalow & Berson's method, other immunological methods appeared, which used the same principle, but in which *the free and* bound I^{131}-labelled *insulin* were separated by *other means* Grodsky & Forsham *(103)* worked out a method in *which the separation* is by precipitation *of the* bound I^{131}-labelled insulin with *sodium* sulphite This method has been *used for* determination of insulin in serum extracts made with acid alcohol With this technique, insulin could not be demonstrated in fasting serum, but on the other hand insulin could be demonstrated in a serum sample drawn 30 minutes after administration of glucose There is thus no doubt that Grodsky & Forsham's method is less sensitive than that of Yalow & Berson, though the reason is not clear It is possible that the relatively low sensitivity in Grodsky & Forsham's method may be due to the fact that the sodium sulphite precipitation results in a poorer separation of free and antibody-bound I^{131}-labelled insulin than is obtained by paper electrophoresis, but the data published do not permit any conclusion to be drawn

Already in 1956 however Skom & Talmage had established that the insulin anti insulin complex in serum could be precipitated if anti globulin was also added *(239)* Various investigators have used this method for the separation of free and antibody-bound I^{131} labelled insulin (*100, 113, 174*) Insulin in fasting serum from normal subjects has been determined by these methods

Unless special precautions are taken, *Yalow & Berson's immunological meth od*, or *methods* based on the same principle, cannot be used for determining the *insulin* in serum from patients who have had insulin treatment This is due to the fact that insulin antibody can be demonstrated in human serum after a certain period of insulin treatment This anti body will influence the insulin–anti in sulin reaction in the test system By ultracentrifugation of serum however, it is possible to sediment the insulin anti body and to determine the antibody free supernatant The amount of im munologically-determined insulin in the supernatant will then constitute a meas ure of the insulin content of the serum Berson & Yalow have further shown that by making high dilutions of serum, it is possible to eliminate the effect of the anti insulin A measurement of the im munologically-determined insulin in highly diluted serum makes it possible to calculate the content in undiluted serum Berson & Yalow (31) have shown that these two methods give identical results

Samols & Ryder (230) have presented a method for simultaneous determina tion of exogenic and endogenic insulin by Berson & Yalow's technique One test system uses human anti insulin prepared

from serum from insulin treated patients This anti insulin has only a very slight affinity for human insulin, and its use for measuring the immunologically active insulin in human serum means that only exogenic insulin is measured, not human insulin Another test system uses *guinea pig anti insulin*, which has a considerable affinity for both human and non human insulin, whereby both exogenic and endogenic insulin are measured The amount of endogenic insulin is thus calculated as the difference between the measurements by the two test systems

The immunological methods described are based on the assumption that the serum insulin which can react with insulin antibody has immunological characteristics identical with those of the insulin extracted from pancreas, which is used for production of the standard insulin solutions Recent investigations have raised doubts as to the validity of this assumption Moloney (172) has thus immunized mice against ox insulin These mice did not develop diabetes, but after injection with mouse pancreas insulin the mice were found to be resistant towards this insulin Injection of anti insulin produced by injection of ox insulin into guinea pigs elicited a diabetic condition in the mice This investigation suggests that serum insulin in mice differs immunologically from purified pancreas insulin It might well be that the immunological difference is due to a change in the insulin molecule, as a result of the extraction processes undergone by the pancreas insulin This assumption gains support from investigations which have shown that cows may form insulin antibody against ox insulin, and that this does not cause diabetes in the animals (213) In view of these findings, it must for the present be considered an open question whether the biologically active serum insulin is immunologically identical with the animal pancreas insulin which is used in the test system

Sensitivity

Insulin amounts greater than 2 5 μU/ml can be determined by Arquilla & Stavitsky s method (13) Berson & Yalow (31) report that they can measure 0 1 μU/ml by their method Insulin amounts greater than 20 μU/ml can be demonstrated by Grodsky & Forsham s technique (103), whereas insulin concentrations greater than 1 μU/ml can be measured by Hales method and by Morgan & Lazarow s method (175, 313)

Precision

The accuracy of the immunological methods is very great, and here these methods are far superior to the biological methods available Morgan & Lazarow (175), in repeated determinations on the same serum, found a standard deviation of 1 % Hales & Randle (115) in two investigations by the immunological method, found lambda values of 0 02 and 0 06

Specificity

Using Berson & Yalow s method, immunologically determined insulin could

tained for the content of immunologically active insulin in the "unknown solution"

Yalow & Berson's method is so sensitive that it can demonstrate insulin in serum. It has been used by other investigators, who were able to reproduce Berson & Yalow's results (230).

After the publication of Yalow & Berson's method, other immunological methods appeared, which used the same principle, but in which the free and bound I^{131}-labelled insulin were separated by other means. Grodsky & Forsham (103) worked out a method in which the separation is by precipitation of the bound I^{131}-labelled insulin with sodium sulphite. This method has been used for determination of insulin in serum extracts made with acid alcohol. With this technique, insulin could not be demonstrated in fasting serum, but on the other hand insulin could be demonstrated in a serum sample drawn 30 minutes after administration of glucose. There is thus no doubt that Grodsky & Forsham's method is less sensitive than that of Yalow & Berson, though the reason is not clear. It is possible that the relatively low sensitivity in Grodsky & Forsham's method may be due to the fact that the sodium sulphite precipitation results in a poorer separation of free and antibody-bound I^{131}-labelled insulin than is obtained by paper electrophoresis, but the data published do not permit any conclusion to be drawn.

Already in 1958, however, Skom & Talmage had established that the insulin-anti-insulin complex in serum could be precipitated if anti globulin was also added (239). Various investigators have used this method for the separation of free and antibody-bound I^{131}-labelled insulin (100, 113, 174). Insulin in fasting serum from normal subjects has been determined by these methods.

Unless special precautions are taken, Yalow & Berson's immunological method, or methods based on the same principle, cannot be used for determining the insulin in serum from patients who have had insulin treatment. This is due to the fact that insulin antibody can be demonstrated in human serum after a certain period of insulin treatment. This antibody will influence the insulin anti-insulin reaction in the test system. By ultracentrifugation of serum, however, it is possible to sediment the insulin antibody and to determine the antibody-free supernatant. The amount of immunologically-determined insulin in the supernatant will then constitute a measure of the insulin content of the serum. Berson & Yalow have further shown that by making high dilutions of serum it is possible to eliminate the effect of the anti-insulin. A measurement of the immunologically-determined insulin in highly diluted serum makes it possible to calculate the content in undiluted serum. Berson & Yalow (31) have shown that these two methods give identical results.

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CHAPTER 2

VARIOUS FORMS OF INSULIN IN SERUM

Shortly after investigators had begun using the insulin assays on blood as described in the last chapter, the various methods were found to give rather divergent results In some of the methods, different physical treatments of plasma apparently caused a considerable rise in its insulin like activity This raised the question whether plasma insulin might not occur in different forms, and whether there might not be a conversion from one form to another, depending on circumstances both *in vitro* and *in vivo* It was conceivable that the insulin was transported in plasma partly as free molecules of insulin, partly in another form perhaps bound in some way to certain plasma protein molecules

To elucidate these circumstances, several investigators examined the insulin activity in different protein fractions separated by precipitation, electrophoresis or resin treatment The results of these investigations are difficult to evaluate, and they are often incompatible because of methodological differences, but a number of investigations nevertheless seem to indicate that insulin is transported in plasma in several different forms.

ILA in serum protein fractions prepared by cold ethanol fractionation and by resin extraction

When serum proteins were separated by Lever's method (148) and the ILA examined by the rat diaphragm technique, several investigators found ILA in fraction I+III (α_2-globulin, β-globulin and fibrinogen) (89, 283), but only Gardiner & co-workers found ILA in fraction II (γ globulins) Fractionation by Lever's method, followed by electrophoresis on treated cellulose, then testing by the rat diaphragm method, showed ILA in albumin α_1-globulin isolated from fractions IV, V, and VI (283) Gardiner & co-workers found no ILA in these protein fractions, but they did not perform an electrophoretic separation As Vargas & co-workers (283) found an insulin antagonist localized to α_1-globulin in fractions IV, V, VI, it is reasonable to believe that the effect of any insulin which may be present in these fractions is inhibited by the antagonist, unless it is removed by additional fractionation

The investigations cited do not give

not be demonstrated in serum from pancreatectomized dogs (74) Immunologically-determined insulin disappears after treatment of serum with cysteine If insulin is added to serum, the immunologically-determined insulin rises by an amount corresponding to the insulin added (31) In normal subjects, ingestion of glucose is followed by a rise in the amount of immunologically-determined insulin in serum (175, 308) With Morgan & Lazarow's method, serum from alloxan-diabetic rats showed lower values for immunologically determined insulin than serum from normal animals (175)

Grodsky & co-workers, using their own technique, found a rise in the values for immunologically active insulin in the pancreatic vein, on infusing a solution of glucose into isolated rat pancreas (104)

It is of considerable interest that Yalow & Berson (310) showed that an insulin derivative, desoctapeptide insulin, which has no biological effect, can be demonstrated by the immunological method, whereas neither the insulin's A chain nor B chain reacts with insulin antibodies Grodsky & co-workers (102) have made similar studies on insulin conversion products They found that insulin treated with carboxypeptidase or dinitro-benzolsulphonate, or insulin which has undergone "mild esterification", can be bound to insulin antibodies This holds also for acetylated insulin and diazotized insulin

By means of their immunological methods, Yalow & Berson (308) and Hales & Randle (113) found that the content of immunologically-determined insulin in serum decreases proportionally with the dilution Examining undiluted rat serum, Morgan & Lazarow (175) obtained very high values for immunologically-determined insulin, lower values were obtained when the serum was diluted This was found to depend on the presence in rat serum of a factor different from insulin, and influencing the test system The effect of this factor seems to disappear on dilution A corresponding phenomenon appears to be lacking in human serum (242)

CONCLUSION

From the results of the above investigations, it seems a reasonable conclusion that there is every probability that other than insulin, normal serum contains no factors which can be registered as insulin-like activity by the rat diaphragm and the rat epididymal fat methods There is likewise every probability that the immunological methods based on Berson & Yalow's principle are specific for insulin in normal serum

No sufficient experimental basis exists for drawing the same conclusion as to the *in vivo* methods or Arquilla & Stavitsky's immunological method

effect on rat diaphragm, it had an insulin like activity when examined by the rat epididymal fat method In a subsequent investigation, the same investigators found that fatty tissue, in contrast to muscle tissue, contains a factor which can activate the hidden insulin-like activity in resin eluates (10), Shaw & Shuey (236) confirmed that this fatty tissue extract can cause a considerable rise in the insulin like activity of serum

Investigations by Anthoniades & co-workers seem to indicate that serum contains different types of ILA one type being active towards rat diaphragm in untreated serum, and another type which has no effect on this tissue, both types can be measured by the rat epididymal fat method Anthoniades put forward the hypothesis that ILA which is adsorbed to the resin, is present in a form bound to a basic protein, and he succeeded in eluting a protein substance from the resin migrating cathodically at pH 7 4 (6) Subsequent electrophoretic investigations (7) however seem to indicate that this basic protein can hardly have significance for the adsorption to the resin

ILA in serum protein fractions prepared by electrophoresis

A few studies throw some light on the occurrence of ILA in electrophoretically separated serum protein fractions Randle & Taylor (209 263) performed electrophoresis on columns of treated cellulose and they determined the ILA by the rat diaphragm method Using this technique, these investigators found ILA in both the albumin α_1 globulin fraction and in the β-γ globulins Lyngsoe (157), by electrophoresis in a polyvinyl chloride block and determination of ILA by the rat epididymal fat method, found ILA in most of the protein fractions examined, though the insulin like activity was distributed into two maxima, corresponding to the serum protein fractions in which Randle & Taylor found ILA Gjedde (58), with a similar technique, found the same distribution of ILA Bolinger & co-workers (33), using the rat diaphragm method, found ILA in the β-globulins, but not in albumin α_1 globulin Other investigators have separated the serum proteins by a method using paper as carrier, and which allows quite large amounts of serum to be separated Insulin like activity was found in the β-globulins, using both an *in vivo* method with intact mice, and the rat epididymal fat method (22, 27) The most important discrepancy between these studies thus concerns the insulin like activity in albumin-α_1 globulin In all those investigations in which ILA was demonstrated in these fractions, the serum proteins were dialyzed at room temperature for more than 24 hours (warm dialysis) after the electrophoresis, a procedure which entails a significant rise in both SILA (97) and ILA in the albumin-α_1 globulin and the β-γ globulin fractions (156) This technique seems not to have been used in other investigations on ILA in electrophoretically separated serum protein fractions, it appears reasonable, there-

sufficient details about ILA in the isolated protein fractions, to permit comparisons with the SILA values which can be demonstrated in unfractionated serum by the same methods. It is of considerable interest, however, that Gardiner & co-workers (89) could demonstrate a pronounced rise in the insulin-like activity localized to γ globulin, after *in vitro* oxygenation of venous blood, but not after oxygenation of venous plasma. These oxygenation tests were inspired by Young's demonstration (312) that SILA (the rat diaphragm method) was higher in plasma prepared from venous blood oxygenated *in vitro*, than in plasma prepared from unoxygenated venous blood. This finding was later confirmed in studies by the rat epididymal fat method (225). These experiments seem to show that γ globulin from non-oxygenated venous blood contains ILA in a "hidden" form, *i.e.* without biological effect *in vitro*, and that this ILA can be activated by oxygenation of whole blood.

In determinations of insulin-like activity in serum proteins using alloxan-diabetic, hypophysectomized rats, Beigelmann, Anthoniades, Goetz, Renold, Oncley & Thorn (26) found ILA in fractions II+III (β- and γ-globulins). In this investigation, the serum proteins were separated by cold ethanol fractionation using methods 6 and 8 (60). By additional fractionation, ILA was found in the β-lipoprotein containing fraction III-0. In the same study, ILA was also found in the fraction IV-4 (albumin-α_1-globulin, α_2 globulin and β_1-globulin). It was found that while there was ILA in fraction II+III isolated from citrated blood, ILA could not be demonstrated in the corresponding protein fractions isolated from blood in which the coagulation had been inhibited by treatment with a cation exchange resin (Dowex 50 or IRC 50). This peculiar result was later taken up for further investigation by Anthoniades & co-workers in numerous studies. Anthoniades (5) showed that insulin-like activity could be eluted from the resin, and electrophoretic separation showed that this ILA was localized to β-γ globulins (7). Gundersen & Anthoniades (107) found that the resin eluates had no insulin-like activity with rat diaphragm, unless they had previously been exposed to a pH considerably higher than physiological. It was found in a subsequent study that in spite of SILA having the same value in untreated and in resin treated serum, ILA could be eluted from the resin (8). In a subsequent study, other investigators have confirmed that considerable amounts of ILA can be eluted from the resin, much greater amounts than the same investigators find in untreated serum (the rat diaphragm method). They have also shown that this ILA is inhibited by anti-insulin (236). These findings seem to indicate that the ILA which can be eluted from the resin, must be present in untreated serum in a "hidden" condition in which it has no effect on rat diaphragm. Gundersen & Anthoniades (107) further showed that while a resin eluate which had been eluted at an acid pH had no

effect on rat diaphragm, it had an insulin like activity when examined by the rat epididymal fat method In a subsequent investigation, the same investigators found that fatty tissue, in contrast to muscle tissue, contains a factor which can activate the hidden insulin like activity in resin eluates (10), Shaw & Shuey (236) confirmed that this fatty tissue extract can cause a considerable rise in the insulin like activity of serum

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fore, to assume that this is why other investigators have not been able to demonstrate ILA in albumin-α_1-globulin

The observation that the insulin like activity in both the albumin-α_1-globulin fraction and the β-γ-globulin fraction will rise following "*warm dialysis*" is of considerable *interest* As *the* ILA is considerably higher in the "warm dialyzed" serum proteins than in untreated serum (157), it may be concluded that the ILA in untreated serum is present in a preponderantly inactive state, which "warm dialysis" changes to an active state On investigating "warm dialyzed" *serum* protein fractions by the rat epididymal fat method, it was found *that the* insulin-like activity, just as is the case of SILA, is partially inhibited by anti insulin A proportion of the very large amount of ILA determined under these conditions, therefore, is undoubtedly insulin, in the sense that it can combine with insulin antibody in an immunological reaction As a certain proportionality was found between ILA which was inhibited by anti-insulin and ILA which was not inhibited, it seems reasonable to assume that both forms of ILA are insulin (156) It must be concluded, therefore, that insulin corresponding to both isolated serum protein fractions is present in two forms, one in which it is active immunologically as well as biologically, and one in which it is only active biologically This is presumably the reason why SILA, in the investigations by the rat epididymal fat method, is only partially inhibited by anti insulin

Studies by *Samaan & co-workers* (229) are of considerable interest in *this* connection These investigators measured SILA in serum with and without the addition of anti-insulin Using this method, they examined the migration of SILA through a membrane which was impermeable to plasma proteins of *high molecular weight, but which was* permeable to purified insulin in solution They *found that SILA* which was inhibited by anti-insulin ("suppressible SILA"—"typical insulin") could penetrate through the membrane, which was impermeable to that part of SILA which was not inhibited by anti insulin ("non-suppressible SILA"—"atypical insulin") This finding seems to indicate that serum insulin which is present in an immunologically active form, is not attached to high molecular proteins, and that the biologically active (with rat epididymal fat) but immunologically inactive part of the serum insulin is present in a higher molecular state than the dissolved insulin Therefore, since ILA which is not inhibited by anti insulin can be demonstrated in both albumin-α_1 globulin and in β-γ globulin it seems reasonable to conclude that both these protein fractions contain insulin in a high molecular state, possibly bound in some way to the plasma proteins

DISCUSSION

It appears from the above that in a few studies, it has been demonstrated that serum contains several forms of insulin like activity Antoniades investigations with the rat diaphragm method see

page 36) have thus shown that in untreated serum, besides the SILA which is immediately demonstrable, there must be a 'hidden' ILA, i e an ILA without any effect on the test system used

Using the rat epididymal fat method, investigators have been able to demonstrate two types of SILA, one which is inhibited and one which is not inhibited by anti insulin (see chapter 1) Gjedde, with the same method, showed that SILA in serum increases after warm dialysis, indicating that in addition to the SILA which can be immediately demonstrated by the rat epididymal fat method, untreated serum contains hidden ILA Lyngsoe s investigations show that hidden ILA is present corresponding both to the albumin-α_1 globulin and to the β-γ globulin fraction

These findings are compatible with the following hypothesis Serum contains two forms of insulin In the one form the insulin is immunologically active in the other form it is immunologically inactive Immunologically inactive insulin is present in two fractions (A' and B) localized by means of electrophoresis to albumin-α_1 globulin and β-γ globulin, respectively

In the rat epididymal fat method, both suppressible and non suppressible SILA can be demonstrated in untreated serum *This seems to indicate* that the method can determine both immunologically active and immunologically inactive insulin SILA which is determined by the rat diaphragm method is totally inhibited by anti insulin, this seems to indicate that in untreated serum the latter method solely determines immunologically active insulin *It has not been possible to* demonstrate that serum contains any insulin which is immunologically active but biologically inactive, it is therefore very likely that the ILA which Anthoniades found was adsorbed to the resin, and which in untreated serum is without any effect on rat diaphragm, is due to immunologically inactive insulin This assumption is in accordance with the fact that ILA which is bound to resin, and which cannot be demonstrated by the rat diaphragm method after elution at acid pH, can be determined by the rat epididymal fat method

As the ILA (the rat epididymal fat method) in serum and in serum protein fractions increases after warm dialysis, serum must contain hidden ILA, as the increase in ILA after warm dialysis can be demonstrated in both albumin-α_1-globulin and in β γ globulin, this suggests that insulin is present in an immunologically inactive form in both these fractions The assumption that immunologically inactive insulin is found in γ globulin gains further support from Gardiner s investigations, which demonstrate that ILA is higher in γ globulin prepared from oxygenated venous blood, than in γ globulin prepared from non-oxygenated venous blood (see page 36) It may be concluded, therefore, that *hidden* ILA, localized to the γ globulin, must be present *in non-oxygenated venous* blood

A possible explanation for the presence of hidden ILA in serum, as demonstrated by resin treatment and by

"warm dialysis", might be that the insulin in serum is inhibited by insulin antagonists (see chapter 3). A considerable part of the hidden ILA which can be demonstrated by the rat epididymal fat method after "warm dialysis", however, is not inhibited by anti-insulin, and this cannot be explained as a consequence of insulin antagonism. Furthermore, as insulin antagonists with an effect on rat epididymal fat are not known in normal serum, it is hardly probable that the presence of hidden ILA (the rat epididymal fat method) is due to insulin antagonism. On the other hand, it cannot be excluded that the hidden ILA (the rat diaphragm method), demonstrable by resin treatment of serum, may be due to insulin antagonism, although it is hardly probable.

From the preceding discussion, it appears that a measure of the immunologically inactive insulin in serum may be obtained by three different methods. This raises the question whether these three methods determine the same or different fractions of the immunologically inactive insulin in serum. Anthoniades (7) reports that ILA which is adsorbed to the resin, migrates electrophoretically so as to correspond to β-γ-globulin. Lyngsoe (158), on treating serum with resin, did not find any definite fall in ILA either in warm dialyzed albumin-α_1 globulin or in warm dialyzed β-γ globulin. It has not been established, however, that it is possible to determine all i.e. immunologically active insulin in serum protein fractions which have been subjected to warm dialysis. Thus the investigation in question does not exclude the possibility that part of the immunologically inactive insulin in the β-γ-globulin fraction is removed following resin treatment of serum. Samaan & co-workers (229), using the rat epididymal fat method, examined suppressible and non-suppressible SILA in serum before and after treatment with resin, and they were unable to demonstrate any fall in the amount of non-suppressible SILA. Neither does this investigation exclude the possibility that part of the immunologically inactive insulin disappears from serum on resin treatment. It is thus possible that the insulin-like activity which can be eluted from the resin, is a measure of the immunologically inactive insulin in the β-γ-globulin fraction.

No studies are available to elucidate whether non-suppressible SILA determined by the rat epididymal fat method, is a measure of the immunologically inactive insulin in albumin α_1-globulin or in β-γ-globulin, or whether it is a measure of both insulin fractions, although the latter possibility seems to be more likely.

As mentioned, the investigations discussed seem to indicate that immunologically inactive insulin is found corresponding to both albumin-α_1 globulin and to β-γ globulin. There are scanty references in the literature to the electrophoretic localization of immunologically active insulin. Anthoniades (7) in a very short report mentions that free insulin (*i.e.* SILA which is not adsorbed to resin) migrates corresponding to al-

bumin α_1 globulin Berson & Yalow (31) examined serum by their immunological method and showed that immunologically determined insulin and added I^{131}-labelled insulin are found on ultracentrifugation to be distributed in the same manner This finding perhaps supports the assumption that the immunologically active insulin in serum, and I^{131} labelled insulin, also migrate electrophoretically in an identical way As I^{131} labelled insulin added to serum migrates corresponding to albumin-α_1-globulin (4, 29, 158 210) it is possible that the immunologically active insulin in serum is found in the same protein fraction It is probable therefore, that albumin α_1 globulin contains both immunologically active and immunologically inactive insulin

Our knowledge of molecular size and possible protein binding of immunologically active and immunologically inactive insulin is very limited The studies of Samaan & co-workers on the diffusion characteristics of suppressible and non suppressible SILA (see page 38) suggest that insulin has a smaller molecular size in the immunologically active form than in the immunologically inactive form

Anthoniades (7) reported that investigations with Sephadex seem to indicate that ILA adsorbed to resin has a molecular weight of more than 40–60,000, whereas ILA not adsorbed to resin has a lower molecular weight These investigations might suggest that insulin in the immunologically inactive form is bound to high molecular proteins, in contrast to immunologically active insulin Other studies, however, tell against so simple an interpretation of the state of serum insulin Berson & Yalow (31), on ultracentrifugation, found only 70 % of the immunologically-determined insulin in the supernatant above the albumin layer Their investigation thus seems to show that a smaller part of the immunologically active insulin in serum is present in a high molecular form

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As mentioned, the investigations discussed seem to indicate that immunologically inactive insulin *is found corresponding* to both albumin α_1 globulin and to β-γ-globulin There are scanty references in the literature to the electrophoretic localization of immunologically active insulin Anthoniades (7) in a very short report mentions that free insulin (*i e* SILA which is not adsorbed to resin) migrates corresponding to al

localized to the albumin fraction The antagonist could be demonstrated in albumin from both diabetic patients and normal subjects, though the investigations suggested that it was found in the highest concentration in diabetics (276, 280) In subsequent studies, Vallance Owen & co-workers also found increased concentration of antagonist in albumin from a majority of patients with recently cured myocardial infarction, in pre-diabetics (patients with a family history of diabetes and abnormal oral glucose tolerance after ingestion of cortisone) (281, 282), and in some relatives of patients with diabetes mellitus (282)

It is of considerable interest that while the antagonist described by Vallance Owen has an insulin inhibiting effect in studies with the rat diaphragm method, concurrent investigations show that it does not influence the insulin action on the rat epididymal fat oxidation of glucose 1 C^{14} to $C^{14}O$ (150, 155)

Vallance Owen & Lilley (280) did not find insulin antagonism in albumin from non substituted adrenalectomized patients whereas albumin from substituted patients was found to be antagonistic Vallance Owen & co-workers 277) found no insulin antagonism in albumin from hypophysectomized patients but Lowy and co-workers in corresponding studies arrived at the opposite result (150) If the patients examined by Lowy had a milder degree of pituitary insufficiency than those examined by Vallance Owen, this would no doubt account for the findings. The publications cited however do not contain the information necessary to support such an assumption

While Lowy & co-workers (150) succeeded in finding insulin antagonism in serum albumin, Keen (132), in a recently published study, has been unable to confirm this finding On the contrary, he found that the extracted albumin contained insulin The reason for the absence of agreement between these studies has not been elucidated It must be emphasized, however, that after the extraction of albumin, Vallance-Owen performs a vacuum extraction which entails considerable foam formation, and which presumably alters a proportion of the protein molecules, where as Keen omitted this procedure This difference of technique may be significant as the most recent studies by Vallance Owen (77) suggest that the B chain of insulin has an insulin antagonistic effect on rat diaphragm, and that the Vallance Owen antagonist has a number of physico-chemical characteristics in common with the B chain Thus, though these findings are compatible with the assumption that the Vallance-Owen antagonist is a break down product of insulin, formed during the extraction procedure, this hypothesis does not explain the insulin antagonism in untreated serum from non ketotic diabetics and the dependence of the insulin antagonist on the adrenal cortex function Further work on these problems will therefore be of the very greatest interest

Field & co-workers, using Stadie s modification of the rat diaphragm meth

CHAPTER 3

SERUM INSULIN ANTAGONISTS

In determinations of SILA before and after the addition of small quantities of insulin to serum, the added insulin is in most cases recovered quantitatively. In corresponding studies on serum from diabetic patients, it was found in some cases that no rise in SILA could be demonstrated after the addition of insulin. It was concluded that the effect of the added amount of insulin was inhibited by the serum. The inhibiting factors were designated insulin antagonists. In the present study, this designation is only used for such serum factors as inhibit the action of insulin on isolated tissue *in vitro*.

Studies published during recent years have demonstrated that insulin antagonists are present in the serum from both normal subjects and diabetic patients. Up till now, 5 insulin antagonists have been described in man, but a closer comparison of these possibly different, possibly identical antagonists, is hampered by the fact that only in a few exceptional cases have investigators attempted to compare their own results with the results of others. It is of considerable interest that insulin antagonism has also been found in diabetic experimental animals, e. g. the rat and the cat.

Later on in the present chapter, a short summary will be given of the significance of insulin antibody as an insulin antagonist in man, although this *anti-insulin is a iatrogenic phenomenon,* without significance for our understanding of the metabolic abnormalities in diabetes mellitus.

Human serum insulin antagonists

Bornstein (36), examining a number of poorly regulated diabetics, found that the serum from these patients lacked SILA, and that when it was injected into ADHA rats, the sensitivity of these animals towards insulin was decreased for up to 6 weeks after the injection.

Vallance-Owen has published numerous studies on serum insulin antagonism. In all these studies, the author and his co-workers used the rat diaphragm method. Using this technique he demonstrated that undiluted serum from poorly regulated, non-ketotic diabetic patients inhibited the action of added insulin, and that the inhibiting effect disappeared after dilution (270, 277). Using trichloracetic acid fractionation (68) and cold ethanol fractionation (60), it was found that the antagonistic effect was

localized to the albumin fraction The antagonist could be demonstrated in albumin from both diabetic patients and normal subjects, though the investigations suggested that it was found in the highest concentration in diabetics (276, 280) In subsequent studies, Vallance Owen & co-workers also found increased concentration of antagonist in albumin from a majority of patients with recently cured myocardial infarction, in pre dia betics (patients with a family history of diabetes and abnormal oral glucose tolerance after ingestion of cortisone) (281 282), and in some relatives of patients with diabetes mellitus (282)

It is of considerable interest that while the antagonist described by Vallance Owen has an insulin inhibiting effect in studies with the rat diaphragm method, concurrent investigations show that it does not influence the insulin action on the rat epididymal fat oxidation of glucose 1 C^{14} to $C^{14}O_2$ (150 155)

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Field & co-workers, using Stadie s modification of the rat diaphragm meth

od (see page 17), found insulin antagonism in serum from patients with diabetic acidosis (81) Field described a number of different characteristics of this antagonist In starch block electrophoresis, it migrated corresponding to the α_1-globulin fraction, it was *not* dialysable, and it was destroyed by boiling or by treatment with chymotrypsin, but not by treatment with *trypsin* (82) The antagonist was not destroyed by repeated freezing and thawing of serum and was *not localized to the* lipoprotein fraction *The antagonist* had no insulinase action, and as it did not inhibit the binding of I^{131}-labelled insulin to rat *diaphragm*, the antagonistic effect is *hardly* due to a binding between insulin and antagonist (81) Field & Rigby (83), using a more sensitive technique *than in the* first studies, found insulin antagonism in serum from a few poorly controlled, non-acidotic, juvenile diabetics with ketonuria In the same study, insulin antagonism was demonstrated in *a few* patients with acute infectious diseases, as well as in three out of four patients with acromegaly and diabetes

Examining serum from acidotic diabetics, later investigators have been able to demonstrate an insulin antagonist which may be identical with the factor which Field characterized in detail Thus, unfractionated serum from such patients will inhibit the insulin effect on the glucose uptake of rat diaphragm (108, 292), whereas no insulin antagonism can be demonstrated in electrophoretically separated serum protein fractions from acidotic diabetics (292) Studies by the rat epididymal fat method on undiluted serum from acidotic diabetics showed a pronounced insulin antagonistic effect, which disappeared, however, *after five fold* dilution of the serum (155) In accordance with this *finding*, Samaan et al (229) were un*able to* demonstrate insulin antagonism in diluted serum from ketotic diabetics by means of the rat epididymal fat meth od

Serum samples from patients with dia betic coma and from a few normal subjects were fractionated by Lever's meth od (148) A factor was found in the α_2-globulin which inhibited the glucose uptake of rat diaphragm (261, 264, 283) The factor is not present in serum from hypophysectomized patients un less the patients are given treatment with growth hormone (264) This factor is presumably insulin antagonistic, like the factors described above, but no studies are available which demonstrate a direct insulin-inhibiting effect

Baird & Bornstein (16) examined se rum from patients with diabetic coma by means *of* an alcohol toluene extraction They found that while the extract con tained SILA, the remainder contained a factor which inhibited the glucose uptake of rat diaphragm In a subsequent study, Bornstein & Hyde (43) found that serum from non-acidotic juvenile diabetics with long term diabetic mani festions contained this inhibiting factor whereas serum from juvenile diabetics without long term diabetic manifestations, but with the same duration of diabetes, lacked the factor It is regret

table that these interesting investigations, which seem to indicate that serum contains an antagonist significant for the development of long term diabetic manifestations, have not been taken up by other investigators.

Recently published studies suggest that an increased concentration of free fatty acids (NEFA) will inhibit the insulin effect on rat diaphragm in vitro (211) This finding makes it reasonable to assume that in serum from certain poorly regulated diabetics without acidosis the insulin antagonism which can be demonstrated by means of the rat diaphragm method may be due to an increased level of NEFA At present there are no studies available on comparisons between NEFA and serum insulin antagonism in diabetics, so that the importance of NEFA for insulin antagonism cannot be considered as proved

As *previously mentioned* (*see page* 22), some studies are available showing that *epinephrine in physiological concentrations* will inhibit the insulin effect on the glucose uptake in rat diaphragm Epinephrine must therefore be characterized as an insulin antagonist (106) Epinephrine has no insulin antagonistic effect on the glucose metabolism of rat epididymal fat (see page 30)

It should be mentioned finally that using both *in vivo* methods the rat diaphragm method and the rat epididymal fat method, it is possible to demonstrate insulin antagonism in serum from diabetics who have developed insulin resistance after having been treated with insulin for some time (61, 79 80, 84, 131, 134, 149, 155, 165, 176, 232, 311) The insulin antagonist has the nature of an antibody, and is localized to the γ globulin or to the inter β-γ globulin fractions (46, 61, 84, 131, 155, 232) Berson and Yalow have by an electrophoretic technique demonstrated insulin antibody in β-γ globulin in all diabetics as early as three months after the start of insulin treatment (29), although the quantity of insulin antibody in serum from insulin resistant *diabetics* is considerably higher than in serum from *insulin sensitive* diabetics (310) Even though serum from patients who have had insulin for a long time, and who are non insulin resistant, is without antagonistic effect on minor quantities of insulin (155), it seems reasonable to assume that the insulin antibody demonstrated by Berson & Yalow causes the insulin antagonism in serum from insulin resistant patients

Serum insulin antagonism in experimental animals

Examining alloxan diabetic rats, Tuerkischer & Wertheimer (265) and Stadie & co-workers (247) found that serum from these animals inhibited the insulin effect on the glycogen synthesis in *normal rat diaphragm* Bornstein & Park (41) found that serum from alloxan-diabetic rats inhibited the glucose uptake in normal rat diaphragm These and later investigations showed that the inhibiting factor was dependent on both pituitary and adrenal glands, as serum from hypophysectomized or adrenalectomized rats did not inhibit the glucose

uptake of rat diaphragm, unless the animals received substitution therapy with growth hormone and cortisone, which were without effect *in vitro*, however (41, 300). In subsequent studies, investigators succeeded in localizing the inhibiting factor to β_1-lipoprotein from both alloxan diabetic rats and normal rats (37, 138). These last-mentioned investigators found that the inhibiting factor reduced the effect of small amounts of insulin on the glucose uptake of rat diaphragm. On the other hand, the antagonist is without any effect on rat epididymal fat (133).

Several investigators have shown that the β_1-lipoprotein-antagonist loses its effect on repeated freezing and thawing of serum (37, 120). This is presumably why an examination of fresh serum from alloxan-diabetic rats shows a decreased SILA, whereas normal values of SILA are found after repeated freezing and thawing of serum (201).

Vallence-Owen & Lukens (275) demonstrated insulin antagonism in serum from pancreatectomized cats. This insulin antagonism has much in common with the β_1-lipoprotein antagonist in rats. For one thing it cannot be demonstrated following hypophysectomy or adrenalectomy. The antagonist effect, however, was not recovered when hypophysectomized pancreatectomized cats had been treated with growth hormone or after adrenalectomized-pancreatectomized cats had been treated with cortisone or hydrocortisone, and the antagonistic effect did not disappear after repeated thawing and freezing of serum.

DISCUSSION

It is not possible to carry out a systematic comparison between the serum insulin antagonists which have been described in man, both normal subjects as well as diabetic patients, and in experimental animals. The studies made are too incomplete for this purpose.

One of the best characterized antagonists is the one described by Field in patients in diabetic acidosis. It has been discussed whether this antagonist, which is localized to α_1 globulin, is identical with the antagonist described by Vallance-Owen in the albumin fraction. An insulin antagonist which is active with rat epididymal fat, however, is present in patients in diabetic acidosis, and as this epididymal fat is not affected by the antagonist described by Vallance-Owen (155), it seems reasonable to assume that Field's and Vallance Owen's antagonists are different. Field's antagonist is not destroyed after repeated freezing and thawing of serum, and it is not localized to the lipoprotein fraction, this antagonist is therefore undoubtedly different in character from that of the lipoprotein antagonist which has been demonstrated in alloxan-diabetic rats. It may not be possible to demonstrate an antagonist in experimental animals analogous to the antagonist in man, as described by Field, but investigations with a view to further characterising the insulin antagonism which can be demonstrated in pancreatectomized cats will also be of considerable interest in this connection.

Bornstein (37) examined lipoproteins in normal *subjects for the purpose of* finding insulin antagonism similar to the type which has been demonstrated in rats He succeeded in demonstrating such an antagonist in one psychotic patient in insulin hypoglycaemia, but not in other normal subjects This interesting finding seems not to have been followed up by other investigators

It is of the greatest interest that serum insulin antagonists which are dependent on hormones have been demonstrated in both man and experimental animals For example, the β-lipoprotein antagonist in the rat, the antagonist in the pancreatectomized cat and perhaps even Vallance Owen s antagonist, all depend on an intact pituitary as well as on intact adrenal glands, while Taylor s α_1 globulin factor in man seems to depend on the pituitary Further investigations are therefore necessary before the possibility of a relationship between these antagonists can be rejected

The demonstration of insulin antagonism in serum from diabetic patients, and in particular Vallance Owen s investigations on the albumin antagonist, have resulted in the phenomenon "insulin antagonism' being introduced into the discussion on the pathogenetic factors in diabetes mellitus The hypothesis has been put forward that an increased production of insulin antagonist in the pre diabetic and the diabetic condition will lead to a surplus production of insulin, which will gradually entail *an exhaustion of the pancreatic tissue*, resulting in secondary insulin deficiency In spite of the fact that there are a number of studies which favour *such a hypothesis, investigations on in*sulin antagonism in animals show that insulin antagonism in serum may occur in situations where a primary insulin deficiency must be assumed, as for example following a pancreatectomy or alloxan treatment If analogies are drawn to patients with diabetes mellitus, then based on the finding of animal experiments it may be supposed that the cause *of the serum insulin antagonism in cer*tain diabetic patients is a reduced production of insulin, although the reverse mechanism is of course also conceivable.

CHAPTER 4

INSULIN CONTENT OF BLOOD FROM NORMAL SUBJECTS AND EXPERIMENTAL ANIMALS

The determination of the insulin content of the blood involves a number of problems For one thing, some of the serum insulin is present in a state of biological and immunological inactivity (see chapter 2), and for another, the serum may contain insulin antagonists which will inhibit the biological effect of the insulin, and thus influence the determination of serum insulin by biological methods (see chapter 3) As a result of these two circumstances, untreated serum examined by biological methods will give a combined measure of the effect of the biologically active insulin and any possible insulin antagonists in the serum, this activity has been designated SILA An examination of untreated serum by immunological methods determines only the immunologically active insulin in the serum But this insulin fraction represents only a very small part of the serum insulin, and should therefore not be considered a measure of the insulin content of the serum Determination of the total insulin content of serum should be made under circumstances in which all the serum insulin is present in an active form, immunologically or biologically If a biological method is used, it should be a condition that the effect of serum insulin antagonists is eliminated The aim of insulin extraction methods is to achieve such circumstances, and using these methods, some investigators have obtained very high values for immunologically determined insulin and biological insulin-like activity in the serum extracts In diluted serum which has undergone prolonged dialysis at room temperature, ILA values have been obtained of an order of magnitude equal to the highest values measured after insulin extraction At present, the values which have been obtained in serum extracts and after "warm dialysis of serum must be considered as the most exact measure of the total insulin content of serum, but it is possible that these methods are unable to activate all the insulin in serum

A large proportion of the insulin in albumin-α_1-globulin (the A fraction) and in β-γ globulin (the B fraction) is found in an inactive condition, presumably bound to serum proteins see chapter 2) Determining the insulin content

of these fractions, therefore, the investigator will experience the same difficulties as in determining the total insulin content of serum The only investigations which have been made on insulin in the A and B fractions, have been carried out by warm dialysis and by using the rat epididymal fat method As previously mentioned, it is not certain that all the insulin in these fractions is determined by this technique, even though the values obtained are very high, since the possibility cannot be excluded that the measurements are influenced by insulin antagonists The activity which is determined by this technique has therefore been designated insulin like activity

A number of studies seem to indicate that serum contains a small amount of immunologically active insulin (see chapter 2) Using the immunological methods for examining serum, only this form of insulin will be determined It is reasonable to assume that the part of SILA which is inhibited after addition of anti insulin to serum, suppressible SILA, is also a measure of the immunologically active insulin

In the published studies on the insulin content of serum, the composition of the normal material has varied in a number of ways. Some investigators have used serum from healthy subjects others have taken it from patients with diseases which presumably do not influence the serum insulin In the present account, persons are considered as normal if they do not suffer from diabetes mellitus or other endocrine diseases. On the other hand, since Western civilizations have a diabetes mellitus incidence of only 1–2 %, and since the incidence of other endocrine diseases scarcely surpasses this figure, material has been accepted as "normal", even though these two patient groups are not explicitly excluded Consequently, obese non diabetic subjects are included in the normal series, in spite of the fact that SILA values in such patients are different from those found in non-obese normal subjects (see chapter 6) This state of affairs must likewise be accepted, for in by far the majority of publications on SILA in normal subjects, it is not possible to distinguish between obese and non-obese subjects

A number of studies are available which provide information on the serum insulin in experimental animals Some of these studies have led to significant information on the secretion of insulin and they are summarized at the end of this chapter

SERUM INSULIN IN NORMAL SUBJECTS

SILA in peripheral venous blood

The insulin like activity determined in untreated blood, serum or plasma by biological methods is designated SILA, whether the investigations have been made with undiluted or with diluted medium

Tables 5 and 6 illustrate the SILA investigations so far published on peripheral venous blood from normal subjects. There are a few investigations on SILA

in blood from other parts of the human circulation, these are summarized on page 62 It appears from tables 5 and 6 that most SILA studies have been on serum, but that a few measurements using *in vivo* techniques and the rat diaphragm method have been done on heparinized plasma. Whether investigations on serum and plasma are comparable has not been established Preliminary results with the rat epididymal fat method seem to indicate that heparin increases the insulin-like activity in serum (168), and heparin appears able to change "complex insulin" (i e ILA which is adsorbed to resin) so that it can be measured by the rat diaphragm method (109)

Comparing SILA in undiluted and in diluted serum, allowance must be made for the fact that after correction for the dilution factor, SILA is higher in diluted than in undiluted medium, both by the rat diaphragm method and the rat epididymal fat method This "dilution effect" was first demonstrated in investigations using the rat diaphragm method (203, 295, 304) Ball & Merrill (19), studying a small experimental series by the rat epididymal fat method (net gas exchange), found a definite dilution effect Subsequent comprehensive studies confirmed this finding (20, 153, 155, 157), although a few minor experimental series showed no statistically significant dilution effect (152, 240, 252) Steinke & co-workers (254), in a subsequent major study, examined diluted serum from one group of normal subjects and undiluted serum from another group of normal subjects. In the study cited, the mean figure for SILA was found to be higher in diluted than in undiluted serum, although the difference was not statistically significant In view of the fact that this study was carried out on two different groups, the result of the investigation does not refute the theory that SILA measured by the rat epididymal fat method is higher in diluted than in undiluted serum

It is not known why SILA is higher in diluted than in undiluted serum It might be considered that dilution results in activation of the immunologically inactive insulin, so that it becomes biologically and immunologically effective This is hardly the case, however, since dilution of serum does not result in a rise in the immunologically-determined insulin (115, 308) The fact that a dilution effect can be demonstrated by the rat diaphragm method was taken to indicate that the effect of one or more insulin antagonists disappears in diluted serum This assumption gained experimental support when Groen & co-workers (106) showed that the insulin antagonistic effect of epinephrine in physiological concentrations, disappears at a dilution where the insulin effect is not lost It is possible, therefore, that the dilution effect which can be demonstrated by the rat diaphragm method is due to an elimination of the insulin antagonistic effect of the epinephrine It may be considered that the dilution effect which can be determined by the rat epididymal fat method, is likewise due

to a 'diluting away' of an insulin antagonist, although in normal serum no insulin antagonists are known which have an effect on this tissue (see chapter 3) It seems more likely, however, that the dilution effect in determinations by the rat epididymal fat method depends on another factor, namely an activation of the immunologically (and biologically) inactive insulin, so that this insulin develops biological activity with respect to fatty tissue Thus, it has been demonstrated that fatty tissue contains a factor which is able to activate 'com plex insulin (107) As large quantities of insulin are present in serum in an immunologically and biologically inac tive condition, it could be conceived that on incubation of fatty tissue with diluted serum, the insulin activating factor would activate part of this insulin

Studies by the rat epididymal fat method have demonstrated a dependence between SILA levels in undiluted and in diluted serum (153) Rising values of SILA in undiluted serum were associated with rising values in diluted serum This finding is compatible with the assumption that the dilution effect is due to a diluting away of an in sulin antagonist but it is also compatible with the hypothesis of an insulin ac tivation as the cause of the dilution ef fect

It appears from table 5 that very few investigations have been made on SILA by in vivo methods These investiga tions were carried out on undiluted se rum heparinized plasma and whole blood The mean values for SILA in these studies vary between 100 and 186 µU/ml in fasting normal subjects It is shown by Bornstein s method that there is a rise in the SILA level after glucose intake.

Most studies by the rat diaphragm method have been made using Vallance-Owen s modification With this method, fairly good agreement has been found between SILA values in undiluted plasma, in fasting normal subjects the mean values vary between 60 and 120 µU/ml A single study, but comprising only 5 subjects, shows lower values (233) Wright (304), using Vallance Owen s technique, found higher values in diluted fasting serum than in undiluted serum, viz a mean value of 200 µU/ml

Groen, Willebrands & co-workers, using other modifications of the rat dia phragm method, found fasting values in diluted serum which were considerably higher than those obtained by Vallance Owen s technique Randle (203) examined both diluted and undiluted plasma from normal subjects whose metabolic state was not known It appears from tables 5 and 6 that Randle s values are significantly higher than those found by Vallance Owen s method, both for fasting serum and for serum samples taken after ingestion of glucose

It appears from table 5 that ingestion of glucose causes a rise in the level of SILA in undiluted venous plasma from normal subjects. Anthoniades & co-workers (9 11, 12) found a rise in SILA level after intravenous administration of glucose in normal subjects.

TABLE 5

SILA in undiluted blood serum or plasma f om normal subjects

Meth od	Med um	Fasting alues		Values after the oral administration of glucose				
				1 hours after glucose		1 -2 hours af er glucose		
		U ml	Mean alue	μU ml	M an alue	μU ml	Mean alue	
In v o								
H A D rats[2])	Blood		100					Gellhorn et al. (1941)[..]
ADH \ rats	Plasma		100(4)		340(4)			Borns ein(1950 [..]
do	do					240–310	340(14)	Borns ein & La rence (1951) [.]
Gl) cogen depo- s t on n rat d a pl ragm	Serum	<100–365	166(20)					Rafaelsen (1961) [..]
Rat diapl agm method								
\ allance O ens mod f cat on	Plasma	0–150	67(15)	0–800	445(17)			\ allance Owen et al (1954)[..]
do	do	0–250	94(18)					Wrigl t (1960 [..]
do	do	45 140	85(15)					Wright (1957)[..]
do	do	11 240	59(12)	71 1000	573(12)			Seltzer & Sm th (1959)[..]
do	do		24(5)					Seltzer (1961)[..]
do	do		30(11)					R zzo et al (1962)[..]
do	do	<100–200	127(12)					Renold et al (1957)[..]
do	Serum	10–400	94(9)					Antl oniades et al (1961 [.] 1962 [.] 1962 [.])
do	do	0 120	48(15)					Sha v & Shuey (1963)[..]
Ra dle s od f cat on	Plasn a	500–6700	2000(5)[1])					Randle (1957)[..]
Rat ep d dymal fat me l od								
Glucose 1 C [14] – CO_2	Serum	33 940	290(38)					Sl eps et al (1960 [..]
	do		290(46)					Ste nke et al (1961 [..]
	do	20–800						Humbel (19 9 [..]
	do	42 182	101(10)			0–520	136(23)	Lyngsoe 1961 [..]
	do	0–290	71(73)					Lyngsoe (1962 [..]
	do	25–245	100(18)	32–460	188(11)			Lyngsoe (1964 [.]
	do		482(16)		86(16)			Hear (19 2 [.]
Net gas excl ange	do	75–100	90(5)					Ball & Merrill 1961 [.]

Tl e f gures in brackets nd ca e tl e nu ber of sera n est ga ed

[1]) Metabol c s ate unkno n

[2]) Hypopl ysecton zed adrenodemedulla ed rats.

TABLE C

SILA in d u d erum o plasma f om normal ub ts

Method	Medium		Fasting values µU/ml	Mean	Values after oral administration of glucose: 1 hour after glucose µU/ml	Mean	1–2 hours after glucose µU/ml	Mean value	
Rat diaphragm method				200 9					Wright (19t0)[104]
Valla e-Owens	Pla a	*							Groen et al (19.2)[44]
cell a ion			63–630 8)						W llebrands et al (19.6)[44]
Ow od f a on	Seru	*	100–3000	1400(14					W llebrands et al (19.8[198]
Ow xl (a o	Seru	/s	300–4600	2300 9)					Ra dle (1954)[44]
O n l fica	Seru n	/ s					9000–22000	13500(7)	Ra d e (19.7)[103]
Ow xl f a n	Plas a	*							
Ow ol f a n	Plas a	* s	3300–5600	3900(.)					
Fat ep l dymal fat method									B ll & Merr ll (1961)[9]
Net gas ex l ge	Seru	/s	173–280	218 5)					do
	l	/ s	) 0–1400	1119(5)					Leake & Burt (1962)[0]
	l	**		750(26)					Ran se r et al (1961)[44]
	do	/s	63–238	152(15)					Burg et al (196.)[4]
	do	/s		193(22)		255(3)			Ran se r et al (1961)[44]
Glu ose up ake	d	1/s	88 233	153(15)					Burg et al (1362)[4]
	l	/s		193 .)		25.(.)			We ges & Worner (1961)[3 0]
	d	1/s	<.0–910(22)						Do sy (1963)[3]
Glu ose l C l- C O2	Seru	/s	107 33	207(9)					Rued et al (1963)[186]
	d	/s	100–800	370(10)	200 1400	630(10)	200 7.0	450(10)	We nges & Worner (1961)[190]
	do	1/s	<.0–910(22)						S e nke et al (1961)[3 4]
	l	*		333(30)					D scl u e t et al (19.9)[44]
	lo	1/s	13.–(8) 15)				230–770	413(6)	I fe ller et al (1961[140]
	d	1/s	290 . 0	458(6)					Ly gsoe (1961)[44]
	lo	1/s	0–43.	139 10)			60–7.0	321(22)	Lyngsoe (1962)[44]
	do	1/s	0–605	171(73)					Lyngsoe (1961)[4]
	d	1/s	.0–300	187(18)	60–6 .	300(11)			D weke (19 3)[44]
	do	1/s	6.–810	377(12)	342 2000	873(12)	120 14.0	501(12)	Sa aa et al (196)[44]
	.	**		718 10)		45.(10)		275(10)	

TABLE 5

SIL A in undiluted blood, serum or plasma from normal subjects

Method	Medium	Fasting values		Values after the oral administration of glucose				
				1 hours after glucose		1½–2 hours after glucose		
		µU/ml	Mean value	µU/ml	Mean value	µU/ml	Mean value	
In vivo								
H A D rats²)	Blood		100					Gellhorn et al (1941)[93]
ADHA rats	Plasma		100(4)		340(4)			Bornstein(1950)[38]
do	do					240–310	340(14)	Bornstein & Lawrence (1951)[40]
Glycogen deposition in rat diaphragm	Serum	<100–365	166(20)					Rafaelsen (1961)[196]
Rat diaphragm method								
Vallance Owens modification	Plasma	0–150	67(15)	0–800	445(17)			Vallance Owen et al (1954)[271]
do	do	*0–250*	*94(18)*					*Wright (1960)*[304]
do	do	45–140	85(15)					Wright (1957)[302]
do	do	11–240	59(12)	71–1000	573(12)			Seltzer & Smith (1959)[234]
do	do		24(5)					Seltzer (1961)[239]
do	do		30(11)					Rizzo et al (1962)[221]
do	do	<100–200	127(12)					Renold et al. (1957)[214]
do	Serum	10–400	94(9)					Anthoniades et al (1961 * 1962 [11] 1962[13])
do	do	0–120	48(15)					Shaw & Shuey (1963)[236]
Randle's modification	*Plasma*	*500–6700*	*2000(5)¹)*					Randle (1957)[205]
Rat epididymal fat method								
Glucose 1 C^{14}– $C^{14}O_2$	Serum	33–940	290(38)					Sheps et al (1960)[237]
	do		290(46)					Steinke et al (1961)[244]
	do	20–800						Humbel (1959)[129]
	do	42 182	101(10)					Lyngsoe 1961 [155]
	do	0–290	71(73)			0–520	136(23)	Lyngsoe (1962)[156]
	do	25–245	100(18)	32–460	188(11)			Lyngsoe (1964 [157]
	do		482(16)		786(16)			Thear (1962 [191]
Net gas exchange	do	75–100	90(5)					Ball & Merrill (1961)[19]

The figures in brackets indicate the number of sera investigated

¹) Metabolic state unknown

²) Hypophysectomized adrenodemedullated rats

of serum which was similar to the one used by Renold's group, Lyngsoe (152) found normal values of an order of magnitude similar to those found by the group mentioned, whereas Gjedde (98), using serum prepared at 4° C, found SILA values which correspond to the values published in Lyngsoe's studies

It is not known why SILA measured by the rat epididymal fat method is dependent on the method for preparation of serum It could be that an activation of the immunologically inactive insulin takes place at room temperature, or that an insulin antagonist is destroyed, or that the immunologically inactive insulin is brought to a state in which it is more easily activated by the insulin activating factor of the fatty tissue (see page 37) It is not known whether SILA determined by the rat diaphragm method also depends on the method for preparation of serum, but it is of interest that investigations using Groen's and Willebrands modifications of this method have all been with serum, whereas the studies using Vallance Owen's modification which gave lower normal values for SILA were made with heparinized plasma

Most studies using the rat epididymal fat method do not give any details for the preparation of serum It is therefore impossible to decide whether the variation in normal values found by different workers merely results from the serum used being prepared in different ways. Reviewing the studies published, however it is of interest to observe that all investigators using Renold's method without modifications (66, 190, 237, 254) obtain almost similar normal values, whereas other groups who have worked out modifications of the rat epididymal fat method, obtain lower and identical normal values (19, 47, 98, 152, 153, 157, 198, 227)

A single material of normal subjects was examined with respect to variations in age and sex (153) No variations were found in undiluted serum, but diluted serum showed a tendency to lower SILA values for women than for men, most pronounced before the 40th year

Investigations using the rat epididymal fat method have shown that SILA rises after ingestion of glucose in normal subjects, both in undiluted and diluted serum (see tables 5 and 6) Following intravenous administration of glucose, a rise is likewise found in the SILA level in both undiluted and diluted serum (47, 159)

The result of the considerations just discussed is that the most likely values for biological SILA in venous serum from fasting normal persons are dependent to only a slight degree on the methods used for measuring SILA In undiluted serum, a great number of investigators find mean values between 50 and 200 μU/ml, whereas higher values are found in diluted serum, the mean values varying between 150 and 200 μU/ml Using all methods, a rise is found in the SILA level in both undiluted and diluted serum, after oral as well as intravenous administration of glucose.

It has not been fully elucidated why SILA determinations by the *different* modifications of the rat diaphragm method show such *varying results* As mentioned previously (page 20), however, it seems reasonable to assume that technical details in the working out of the rat diaphragm method play a significant part In this connection it might be *emphasized* that in none of the modifications of this method, is a "carrier protein" used in the standard insulin solutions As a result, part of the insulin in these solutions may be adsorbed to the glass-ware, and the real insulin concentration in the standard insulin solutions will therefore be less than intended Consequently, comparisons of the insulin-like-activity of plasma with the effect of the standard insulin solutions may result in too high values being determined for the insulin-like activity of plasma It seems reasonable, therefore, to take the lowest normal values obtained by the rat diaphragm method, as being the most correct measure of SILA by this technique Randle's own investigations (199) support the hypotesis that his results can hardly be a real measure of the SILA level in normal untreated plasma For example, he found that a plasma which showed a SILA value of 13,000 μU/ml by the rat diaphragm method, had no hypoglycaemic effect on alloxan-diabetic hypophysectomized rats When insulin solutions with 2000 μU/ml were injected into these animals, they reacted with a pronounced fall in blood sugar

As a result of the considerations mentioned above, the normal values of SILA obtained by Shaw & Shuey (236) using Vallance Owen's modification of the rat diaphragm method must be considered the most correct values at present. These *investigators added* gelatine to the standard insulin solutions, and, as was anticipated, they found lower values of SILA than did other investigators, using Vallance Owen's modification without any addition of gelatine

SILA determinations in peripheral venous serum using the rat epididymal fat method have given normal values which vary widely, although not so much as the results with the rat diaphragm method (tables 5 and 6) The cause of this variation has not been elucidated, but it is presumably due to technical details in connection with the preparation of the serum, and perhaps also with the details of the procedure Lyngsoe has thus shown that by preparing serum from blood which is kept cooled to 4° C, lower SILA values are obtained than if the blood coagulates at room temperature (98, 152) Thawing frozen serum at room temperature also results in an increase in the SILA level (152), but not if the serum is thawed at 4° C (98) Renold's group made investigations using serum which had been prepared at room temperature and subsequently frozen, whereas Lyngsoe's investigations were made on fresh serum prepared at 4° C It seems likely therefore, that the difference between the normal values in the two sets of results can be explained as a consequence of these conditions Using a technique for the preparation

of serum which was similar to the one used by Renold's group, Lyngsoe (152) found normal values of an order of magnitude similar to those found by the group mentioned, whereas Gjedde (98), using serum prepared at 4° C, found SILA values which correspond to the values published in Lyngsoe's studies

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Immunologically active insulin in peripheral venous blood

Tables 7 and 8 show the studies made on the occurrence of immunologically active insulin in venous serum. In fasting serum, the immunological methods have given normal values amounting to about 20 μU/ml, although Spellagy & Goetz (243), using Goetz & Greenberg's method, obtained somewhat higher values. Studying the amount of SILA which is inhibited by the addition of anti-insulin ("suppressible SILA"), the majority of investigations by the rat epididymal fat method arrive at values which are about twice as high as those found by examination of immunologically active insulin using immunological methods. A single study, however, gives a mean value of 13 μU/ml. It is of considerable interest that the investigations cited do not show values for suppressible SILA which are higher in diluted than in undiluted serum. As mentioned previously, the investigations using immunological methods do not show any dilution effect (113, 308).

All studies show a rise in both immunologically determined insulin and suppressible SILA following oral and intravenous administration of glucose (see tables 7 and 9). Considering the fact that the two methods for determining immunologically active insulin in serum are quite different in principle, it is surprising to find so little difference in the mean values quoted for the normal series examined. This strongly suggests identity between the part of the serum insulin determined by the immunological methods, and the part determined as suppressible SILA.

Since studies by the rat diaphragm method have shown that addition of anti insulin to serum produces a complete inhibition of SILA, it is reasonable to assume that using this method, SILA

TABLE 7

Immunologically determined insulin in serum from normal subjects

Fasting values		Values after the oral administration of glucose				
		1 hour after glucose		1½–2 hours after glucose		
μU/ml	Mean value	μU/ml	Mean value	μU/ml	Mean value	
2– 66	21(30)	18–342	139(30)	21–233	106(30)	Yalow & Berson (1960)[260]
0– 70	23(30)					Samols & Ryder (1961)[220]
2– 63	19(100)					Samols & Marks (1963)[221]
0– 27	17(5)	20–100	49(5)			Hales & Randle (1963)[114]
0– 50	24(10)	25–170	80(10)	25–150	70(10)	Ruedi et al. (1963)[223]
11– 50	34(12)					Orskov (1963)[illegible]
30–115	62(20)					Spellagy & Goetz (1963)[243]

The figures in brackets indicate the number of sera investigated

TABLE 8

Suppressible SILA and Non-suppressible SILA in normal subjects

Method	Dilution	Suppressible SILA				Non-suppressible SILA				
		Fasting values		Value after the administration of glucose		Fasting values		Value after the administration of glucose		
		µU/ml	Mean value	µU/ml	Mean value	µU/ml	Mean value	µU/ml	Mean value	
Glucose uptake + rat gas exchange	1/4	0-157	54			53-238	106			Ramsey et al (1961)[18]
	1/4	0-93	13-22			70-380	180(22)			Burg et al (1962)[4]
	1/10	0-40	13(5)	50-170	111(5)[1]	70-190	120(5)	60-200	131(5)[1]	do
	1	0-65	35(5	0-600	300(5)[3]	55-260	170(5)	70-350	200(5)[3]	do
Glucose 1-$C^{14}O_2$	1/4	0-200	53(10)	80-670	270(10)[2]	110-250	165(10)	100-370	185(10)[2]	Samaan et al (1962)[32]
	d		43(10)		188(10)[2]		164(10)		165(10)[2]	Samaan et al (1963)[334]
	Undiluted	12-122	45(17)	33-440	151(17)[4]	0-210	82(17)	9-140	75(17)[4]	Lyngsoe & Lundbæk (1965)[149]

[1]) 60 minutes after the oral administration of glucose (Approximate values)
[2]) 1 0 minutes after the intravenous administration of glucose (Approximate values)
[3]) 60 minutes after the oral administration of glucose
[4]) 10 minutes after the intravenous administration of glucose
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0– 27	17(5)	20–100	49(5)			Hales & Randle (1963)[113]
0– 50	24(10)	25–170	80(10)	25–150	70(10)	Ruedi et al (1963)[224]
11– 50	33(12)					Orskov (1963)[212]
30–115	62(20)					Spellagy & Goetz (1963)[243]

The figures in brackets indicate the number of sera investigated

TABLE 9

Immunol gual y determ n d insulin and ILA in s un e a ts f o n normal ubjec

A ethor	M h of action	Fas g u s		Va es af er dn n a of g cose		
		μU m	M an	μU m	M ai al	
A D A n e	Own ne l od	800–1150	950(3	2800–3700	2960(5)	Ba rd & Borns e n (1959)
A ju lla & S a tsky	Ba rd & Borns e n	2000–50000	14200(11)	2500–2 0000	104500(5)	Pellegr n e al (1958)[44]
do	do	500–62000(5)				Andrean et al (1960)[4]
d	do	2000–25000	13700(5)			Rod r (1960)[111]
Own n n u olog c ne l od	A al e hanol		20(6)	10–80	31(5)	Grodsky et al (1960)[103]
lo.	do	20–82	40(8)	20–130	80(3)	Karam et al (1963)[10]
Rat d apl agr me hod (Vallan e Owens od f ca io)	Tl e res n n e l od	0–1000	427(9)	0–300	73(9)*)	Antl on ades et al (1961* 1962 1962 *)
do	do	230–1130	616(10)			Sl aw & Sl uey (1963)[104]

) M enalectu ed al oxa -d abe c nu e
*) I es ga ed 10 n nu es after tl e in ra e ous ad nis a o of glucose (Approx n a e values)
Tl fgu es b a kets l a e tl e nu nber of sera n es ga ed

is a measure of the overall effect of the immunologically active insulin and insulin antagonists in serum Using what must be considered the best modification of the rat diaphragm method (236), SILA values are found which correspond to the values found for suppressible SILA in most investigations by the rat epididymal fat method

Immunologically inactive insulin in peripheral venous blood

As previously mentioned (chapter 2) it appears that immunologically inactive insulin in serum may be measured by three different methods 1) By adding anti-insulin to serum and determining non-suppressible SILA by the rat epididymal fat method 2) By examining ILA in serum and serum protein fractions after warm dialysis 3) By treatment of serum with resin and elution of ILA from the resin The second of these three methods, *viz* warm dialysis of electrophoretically separated serum protein fractions, makes it possible to measure two fractions of immunologically inactive insulin in serum, the A fraction, which is localized to albumin-α_1-globulin, and the B fraction, which is localized to β-γ-globulin It is possible that resin treatment and determination of ILA in the eluate provides a measure of the immunologically inactive insulin localized to β-γ-globulin, but at present this problem remains unclarified

The only quantitative studies published on the two fractions of immunologically inactive insulin in serum, the A fraction and the B fraction, were made by electrophoretic separation of the serum protein fractions followed by warm dialysis As previously mentioned (page 41), it is probable that both immunologically active and immunologically inactive insulin are determined by examining the albumin-α_1-globulin fraction with this technique Since the amount of immunologically active insulin is but a negligible part of the insulin content of this protein fraction, it is justifiable to ignore it, and to consider ILA in warm-dialyzed albumin α_1 globulin as a measure of the immunologically inactive insulin in this fraction

It might be that part of the immunologically inactive insulin in serum could be isolated by extraction with acid alcohol Studies of serum extracts by Grodsky, Forsham & Karam (*103, 130*), do not appear to support this assumption, as the values by this method were not significantly higher for immunologically determined insulin in the extract than those usually measured in untreated serum by the immunological methods (see table 9) Baird & Bornstein (16, 17) however, extracting serum by means of alcohol-toluene and determining ILA in the extract by the use of an *in vivo* method, found values that were considerably higher than those which may be demonstrated by a similar method in untreated serum (tables 5 and 9) It has also been shown that extract prepared in the same way contains considerable amounts of immunologically active insulin, determined by Arquilla & Stavitsky's method (see table 9) These

reasonable to suppose that all the methods determine only a greater or lesser portion of a large 'pool' of immunologically inactive insulin

Several studies elucidate the changes in immunologically inactive insulin after ingestion of glucose. Using Baird & Bornstein's extraction technique, a rise in ILA was found in the extract (table 9) In warm dialyzed serum protein fractions a rise in ILA was found in the albumin α_1 globulin fraction (table 10) which presumably reflects a rise in the immunologically inactive insulin in this protein fraction (157) In untreated serum, on the other hand, no rise was found in the amount of non suppressible SILA after ingestion of glucose (table 8) Non suppressible SILA determined in serum which has not been fractionated by electrophoresis presumably constitutes only a small part of the immunologically inactive insulin in serum It seems reasonable, therefore, to assume that the results obtained by this technique reflect only to a slight degree the changes which occur in the amount of immunologically inactive insulin From the available studies it seems highly probable that the amount of immunologically inactive insulin in peripheral venous blood will rise after ingestion of glucose presumably on account of a rise in the insulin in albumin-α_1 globulin

A few investigators have examined immunologically inactive insulin in serum prior to and after intravenous administration of glucose Burgi et al (table 8) found no change in non suppressible SILA 120 minutes after administration Anthoniades & co-workers (table 9) found a fall in the 'complex insulin', i e insulin which can be eluted from the resin, 10 minutes after administration of glucose. Lyngsoe & Lundbæk (table 8) likewise found no change in non suppressible SILA 10 minutes after glucose, just as ILA in the B fraction was unchanged (table 10) A fall was nevertheless found in ILA in the A fraction In spite of this fall not being quite significant statistically, it is of considerable interest in the light of Anthoniades results These investigations by means of two different methods for determining the immunologically inactive insulin, suggest that a fall in insulin may occur soon after intravenous administration of glucose. Although Anthoniades preliminary results seem to indicate that insulin which is adsorbed to resin migrates electrophoretically like β-γ globulin (see page 40), the possibility cannot be altogether excluded that the two series of investigations reflect a change in one and the same fraction of the immunologically inactive insulin

SILA immunologically active and immunologically inactive insulin in blood from the portal vein, hepatic vein and peripheral arteries

In normal subjects, there are only a few investigations on the insulin content of blood not originating from a peripheral vein

Humbel & co-workers (121) examining SILA (rat epididymal fat method) in the hepatic vein and the brachial

values for immunologically determined insulin in the serum extracts are considerably higher than values found by means of other immunological methods in untreated *serum* (see table 8). It must be considered likely, therefore, that immunologically inactive insulin in serum can be extracted by Baird & Bornstein's extraction method, but no studies are available which elucidate whether the extracted insulin derives from both or only one of the two previously described fractions of immunologically inactive insulin.

In contrast to previous methods, it seems possible that Davidson's recently published technique of acid alcohol extraction of serum will permit extraction of immunologically inactive insulin (65). It is stated that up to 7000 μU/ml serum has been demonstrated by this method, *i. e.* significantly more than corresponds to the amount of immunologically active insulin in serum. Davidson's method employs prolonged dialysis of the acid alcohol extract at 1° C, *in* contrast to other methods for insulin extraction, and preliminary studies suggest that this is the reason why Davidson's method gives higher values in the extract than the extraction methods so far used.

Tables 8, 9 and 10 show studies elucidating the occurrence of immunologically inactive insulin in serum. It appears from the tables that the normal values for non-suppressible SILA determined by the rat epididymal fat method, are somewhat lower than the ILA values which were found by Anthoniades' resin technique using the rat diaphragm method. The normal values determined in warm dialyzed serum protein fractions, and found after extraction by Baird & Bornstein's method, are significantly higher than the ILA values found after resin treatment and as non suppressible SILA. It is not known why the techniques mentioned for determining immunologically inactive insulin have given so varying results, but it seems

TABLE 10

ILA in warm dialyzed A fraction and B fraction from normal subjects

A fraction				B-fraction			
Fasting values		Values after the administration of glucose		Fasting values		Values after the administration of glucose	
μU/ml	Mean value	μU/ml	Mean value	μU/ml	Mean value	μU/ml	Mean value
390–7200	1900(18)	300–10800	3142(11)	560–13000	2820(18)	760–12400	3685(11)[1]
260–5800	1740(18)	200–4800	1300(15)	320–6100	2530(18)	500–4500	2270(15)[2]

[1]) Lyngsoe (1964)[157]. The normal subjects were investigated in the fasting state and 60 minutes after the oral administration of glucose.

[2]) *Lyngsoe & Lundbæk (1965)*[158]. *The normal subjects were investigated* in the fasting state and 10 minutes after the intravenous administration of glucose.

The figures in brackets indicate the number of sera investigated.

found values between 5 and 12 μU/ml (mean value 9 μU/ml) in 4 subjects These values are somewhat lower than the values which are usually found by Yalow & Berson's method for immunologically-determined insulin in peripheral venous plasma (see table 7)

SERUM INSULIN IN EXPERIMENTAL ANIMALS

SILA, immunologically active and immunologically inactive insulin in peripheral venous blood

It appears from table 11 that the mean SILA values found by various investigators in fasting experimental animals corresponded in order of magnitude to those found in normal subjects by the same methods. There is only one exception to this rule, Steinke & co-workers (257) finding of an average SILA value of 106 μU/ml (rat epididymal fat method) in undiluted cat serum, whereas an average value of 290 μU/ml was found in fasting normal subjects (table 5)

Studies on the occurrence of immunologically active insulin in serum from experimental animals indicate that rats have higher fasting values of immunologically-determined insulin than humans 116 175) On the other hand values of immunologically-determined insulin in fasting dogs were found to correspond to the values in humans.

The amount of immunologically inactive insulin in blood from experimental animals has been elucidated both by extraction studies and by determination of non suppressible SILA by the rat epididymal fat method These investigations, too, show values in fasting experimental animals which correspond to the findings in human blood (tables 8 and 9)

Samaan et al (226) found a rise in both suppressible and non suppressible SILA after intravenous administration of glucose in three dogs This finding differs from corresponding studies on normal subjects, which all showed an unchanged non suppressible SILA level after intravenous administration of glucose (table 8) As the study comprised only a few animals, however, it is not possible to conclude from it that immunologically inactive insulin in humans and in dogs reacts differently after intravenous administration of glucose.

SILA immunologically active and immunologically inactive insulin in blood from the pancreatic vein and the hepatic vein

Studies on the insulin content of blood from the pancreatic vein and the hepatic vein have only been made in dogs. Using the rat diaphragm method and the rat epididymal fat method, several investigators have shown that the SILA level in blood from the portal vein and the pancreatic vein is higher than the SILA level in peripheral venous blood and blood from the hepatic vein The same investigators showed that SILA in blood from the pancreatic vein rises after the administration of glucose (74, 170

vein, found the highest SILA level in the hepatic vein

Using Vallance-Owen's modification of the rat diaphragm method, Baird & Farquahar (18) found that plasma from the portal vein in the new-born had a mean SILA value of 200 μU/ml Five minutes after intravenous administration of glucose there was a fall in the SILA level to an average value of 72 μU/ml!

Anthoniades et al (11), using the rat diaphragm method, examined SILA and ILA in resin eluates of blood from the portal vein In fasting normal subjects, SILA varied between 200 and 2000 μU/ml, whereas the mean value for ILA in the resin eluates corresponded to 50 μU/ml After intravenous administration of glucose, SILA rose in 3 out of 4 normal subjects, while the ILA values in the resin eluates remained unchanged

Using the same technique, the same investigators (12) examined blood from the hepatic vein In two fasting normal subjects, SILA was found to be 50 and 450 μU/ml, respectively, whereas the values for ILA in the resin eluates corresponded to 550 and 500 μU/ml serum Following intravenous administration of glucose, SILA rose while ILA in the resin eluates fell in both the normal subjects

Samols & Ryder (230), in an excellent study, used Yalow & Berson's method for investigation of immunologically-determined insulin in the hepatic vein An average of 24 μU/ml was found in 7 subjects with porto-caval anastomosis or thrombosis of the portal vein This value is slightly higher than the mean value found by Samols & Ryder in peripheral venous blood from fasting subjects In the same study, immunologically determined insulin was measured in blood from the femoral artery The mean value for immunologically determined insulin in serum from the femoral artery was found to be 26 μU/ml, and higher than the normal value in serum from peripheral veins Samols & Ryder examined the concentration of both immunologically-determined endogenous insulin and of immunologically-determined exogenous ox insulin in the hepatic vein, femoral vein and peripheral arteries They concluded from their results that an uptake of both endogenous and exogenous insulin occurred in both the liver and the lower extremities This conclusion is correct, provided that no change takes place from immunologically active to immunologically inactive insulin in the liver or the lower extremities, but investigations made by Samaan & co-workers (226, 227) seem to indicate that this possibility can hardly be excluded

Rizzo & co-workers (220) have compared SILA (the rat diaphragm method) in the femoral artery and vein They found a higher SILA level in arterial plasma than in venous plasma, after ingestion of glucose Ruedi et al (225), using the rat epididymal fat method, found corresponding conditions in fasting normal subjects

Rabinowitz & Zierler (193) examined arterial plasma from fasting normal subjects by Yalow & Berson's method They

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Samaan et al (226) found a rise in both suppressible and non suppressible SILA after intravenous administration of glucose in three dogs This finding differs from corresponding studies on normal subjects, which all showed an unchanged non suppressible SILA level after intravenous administration of glucose (table 8) As the study comprised only a few animals however, it is not possible to conclude from it that immunologically inactive insulin in humans and in dogs reacts differently after intravenous administration of glucose.

SILA immunologically active and immunologically inactive insulin in blood from the pancreatic vein and the hepatic vein

Studies on the insulin content of blood from the pancreatic vein and the hepatic vein have only been made in dogs Using the rat diaphragm method and the rat epididymal fat method, several investigators have shown that the SILA level in blood from the portal vein and the pancreatic vein is higher than the SILA level in peripheral venous blood and blood from the hepatic vein The same investigators showed that SILA in blood from the pancreatic vein rises after the administration of glucose (74, 170

TABLE 11

Determination of insulin in peripheral venous blood from experimental animals

	Method	Mean fasting value µU/ml	
Rat	Randle's modification of the rat diaphragm method	14600	v Holt et al (1957)[133]
	Own immunologic method	121(15)	Hales & Kennedy (1964)[114]
	Own immunologic method	104	Morgan & Lazarow (1963)[1 5]
	Acid ethanol extraction + Arquilla & Stavitsky's immunologic method	17400(10)	Pellegrini et al (1958)[185]
	Baird & Bornstein extraction + Arquilla & Stavitsky's immunologic method	1800–18000	Arquilla et al (1960)[14]
	Randle's modification of the rat diaphragm method	16000(5)	Randle & Young (1956)[204]
	Rat epididymal fat method	125(22) diluted $^1/_5$	Doisy (1963)[73]
Dog	Hypophysectomized adrenodemedullated rats	100	Gellhorn et al (1941)[92]
	Own modification of the rat diaphragm method	270(10) diluted $^1/_{20}$	Takeuchi et al (1957)[259]
	do	288(8) diluted $^1/_{20}$	Takeuchi et al (1961)[60]
	Vallance Owens modification of the rat diaphragm method	64(3)	Candela et al (1958)[43]
	do	160(13) arterial plasma	Metz (1960)[1 0]
	Rat epididymal fat method	610(9) diluted $^1/_8$	Egdahl & Goldberg (1962)[4]
	do	48(3) suppressible SILA diluted $^1/_5$	Samaan et al (1962)[226]
	do	99(3) non suppressible SILA diluted $^1/_5$	do
Cat	Randle's modification of the rat diaphragm method	2400(5)	Randle & Young (1956)[204]
	Vallance Owens modification of the rat diaphragm method	119(5)	Vallance Owen & Lukens (1957)[2 4]
	Rat epididymal fat method	106(6)	Steinke et al (1962)[237]
Pig	Rat epididymal fat method	111(5) 510(5) diluted $^1/_5$	Lyngsoe (1962)[158]
Cow	Baird & Bornstein extraction + own modification of the rat diaphragm method	260(12)	Cunningham (1962)[44]
Sheep	do	300(12)	Cunningham (1962)[44]
Monkey	Rat epididymal fat method	97(11) undiluted 198(11) diluted $^1/_8$ 795(11) diluted $^1/_{15}$	Ball & Knobil (1963)[20]

The figures in brackets indicate the number of sera investigated

187, 226, 234) Metz (170), using the rat diaphragm method, showed that there is a proportionality between the serum glucose level and the SILA values in blood from the pancreatic vein

Samaan & co-workers (226) have made a thorough investigation of the changes in suppressible and non suppressible SILA in blood from the pancreatic vein, hepatic vein and femoral vein in dogs following the administration of glucose They found that the values for suppressible SILA in the pancreaticoduodenal vein were significantly higher than the values for non suppressible SILA in both fasting animals and after administration of glucose Both before and after administration of glucose much higher values of suppressible SILA were found in the pancreaticoduodenal vein than in the hepatic vein and femoral vein The values for non-suppressible SILA were identical in the three types of venous blood while fasting After administration of glucose a rise in non suppressible SILA was seen in all three veins but the rise was most pronounced in the femoral vein

In a subsequent study Samaan & co-workers (229) showed that on infusion of large quantities of dog and ox insulin into the portal vein in pancreatectomized dogs there was a moderate rise in non suppressible SILA and a considerable rise in suppressible SILA in the hepatic vein The same investigators found a fall in non suppressible SILA in peripheral venous blood on infusion of insulin into the femoral vein in two pancreatectomized dogs, whose liver had been excluded from the blood circulation On these findings, Samaan & co-workers proposed the hypothesis that non-suppressible SILA is formed in the liver This hypothesis agrees with the finding of reduced values of non suppressible SILA in peripheral venous blood, in patients with cirrhosis of the liver (227)

Burgi & co-workers (48), perfusing isolated rat liver with rat insulin, were unable to demonstrate any rise in non-suppressible SILA during the perfusion

DISCUSSION

It appears from the foregoing that a number of studies of peripheral venous blood elucidate the changes in immunologically active and immunologically inactive serum insulin after administration of glucose These studies have shown that the immunologically active insulin, determined as suppressible SILA or immunologically-determined insulin, increases after both intravenous and oral administration of glucose Most studies on peripheral venous blood suggest that ingestion of glucose is followed by a rise in immunologically inactive insulin, and this probably reflects a change in the A fraction A few studies suggest that the immunologically inactive serum insulin will fall soon after intravenous administration of glucose, possibly reflecting a fall in the A fraction No change in the B fraction has been observed, either after intravenous or oral administration of glucose These findings show that the different forms and frac-

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DISCUSSION

It appears from the foregoing that a number of studies of peripheral venous blood elucidate the changes in immunologically active and immunologically inactive serum insulin after administration of glucose These studies have shown that the immunologically active insulin, determined as suppressible SILA or immunologically determined insulin, increases after both intravenous and oral administration of glucose Most studies on peripheral venous blood suggest that ingestion of glucose is followed by a rise in immunologically inactive insulin, and this probably reflects a change in the A fraction A few studies suggest that the immunologically inactive serum insulin will fall soon after intravenous administration of glucose, possibly reflecting a fall in the A fraction. No change in the B fraction has been observed, either after intravenous or oral administration of glucose. These findings show that the different forms and frac-

tions of serum insulin react differently when the blood sugar level changes, so that it is reasonable to ascribe different physiological significance to them

Studies in both man and experimental animal suggest that the concentration of immunologically active insulin is greater in the pancreatic vein and the portal vein than elsewhere in the circulation (see pages 62, 63, and 65) Examination of pancreatic venous blood has also shown that SILA measured by the rat diaphragm method rises in proportion to the blood sugar level (170) These investigations agree with the assumption that immunologically active insulin is formed in the pancreas, and that the production is regulated by the blood sugar level

Studies in man indicate that the concentration of immunologically active insulin is higher in the peripheral arteries than in the peripheral veins (see page 62) No investigations are available which elucidate whether this is due to a peripheral uptake of insulin, or to a change from an immunologically active form of insulin to an immunologically inactive form

A few studies seem to suggest that the concentrations of non suppressible SILA and "complex insulin" (i e insulin which can be eluted from resin), respectively, are lower in the portal vein than in the hepatic vein (see page 62 and page 65) These findings support the assumption that immunologically inactive insulin is formed in the liver, a concept compatible with the investigations on liver "insulin uptake" by Samols & Ryder (see page 62)

In all the studies of serum from the portal vein and the hepatic vein, the immunologically inactive insulin has been determined by methods which show only a small proportion of this form of insulin, and which do not distinguish the A and B fractions In view of this, it must be concluded that *for* the time being, these studies do not permit a decision as to the site of formation of immunologically inactive insulin in the normal organism

CHAPTER 5

INSULIN CONTENT OF BLOOD FROM PATIENTS WITH DIABETES MELLITUS

Most studies on serum insulin in patients with diabetes mellitus show serum levels which vary according to the type of diabetes mellitus involved. These studies are difficult to compare as the patient series are often classified on the basis of different clinical characteristics and in many cases the clinical information is so scanty that the patients cannot be grouped in any other way than that followed in the original publication. In the present study, an attempt has been made to systematize these investigations by subdividing patients with diabetes mellitus into two groups: those with acidosis and those without acidosis. The latter group has been subdivided further into different types.

On reviewing the studies made on poorly regulated diabetic patients, it was found that some of the patients had been examined when they had high blood sugar level and considerable ketonuria but that information on serum pH or serum bicarbonate was lacking. It was decided to regard these patients as not having acidosis unless it was stated that the patients were in diabetic acidosis, precoma, or coma.

In the present survey of serum insulin investigations on non-acidotic diabetic patients, an attempt has been made to classify the patients examined into 3 groups: 1) juvenile diabetics, comprising all young patients, together with those older diabetic patients whose disease—as in the case of the young diabetics—is accompanied by heavy glycosuria and a tendency to ketosis. 2) Older non-obese diabetics. 3) Older obese diabetics. In this grouping, the designations 'juvenile' and 'older' refer to patient age on diagnosis of the diabetes mellitus. On this classification, the group 'older non-obese diabetics' includes only older patients with a 'mild' type of diabetes mellitus, i.e. without ketonuria and without pronounced glycosuria. Many investigations on older diabetics without obesity, however, state nothing as to the patient's tendency to ketosis. In the present study, it has been chosen to classify such patients as 'older non-obese diabetics', since patients with a tendency to ketosis constitute a minority within the group of older diabetics without obesity. On similar grounds, studies on obese diabetics whose

age is not stated have been grouped together with the studies on "older obese diabetics"

It will appear from the above that these attempts at grouping the patients who are reported in the literature, are far from certain It is regrettable that so many of the publications on plasma insulin do not meet the simplest requirements for clinical description of patient material In many otherwise excellent and extensive studies, therefore, it is only with difficulty and considerable uncertainty that the results presented can be included in the discussion on the pathophysiology of diabetes mellitus

SERUM INSULIN IN NON ACIDOTIC PATIENTS WITH DIABETES MELLITUS

SILA in peripheral venous blood

Investigations by means of the ADHA-rat method, and most investigations by the rat diaphragm method, show either no SILA or low SILA values in patients with juvenile diabetes mellitus It appears from table 12 that those investigators who have been unable to demonstrate SILA in serum from juvenile diabetics, have examined undiluted serum, whereas in the remaining studies, SILA was examined in diluted serum The difference found between determinations on diluted and undiluted serum is probably due to the presence of an insulin antagonist which is without any effect in diluted serum (276), although at present it is not possible to establish which of the insulin antagonists described is responsible

Examination of SILA in juvenile diabetics by means of the rat epididymal fat method shows a different pattern Normal fasting SILA values are found in some studies (78, 154, 155), while elevated values have been demonstrated in other studies (66, 189, 253, 254, 255) The cause of these divergencies is unknown The increased SILA in the juvenile diabetics studied by Daweke (66) may well be due to exogenous insulin, but this cannot be the reason for the high SILA values in Steinke's investigations (253, 254, 255) as these latter determinations were made exclusively on untreated patients More recent investigations, however, seem to indicate that fasting serum from juvenile diabetics contains normal quantities of immunologically active insulin (see page 70), but increased quantities of immunologically inactive insulin (see page 77) It might be assumed, therefore, that those modifications of the rat epididymal fat method used by Steinke & Daweke, determine immunologically inactive serum insulin to a greater degree than is the case with the modification used by Lyngsoe & co-workers Support for this assumption is gained from the fact that the normal values for fasting SILA found by the two first-mentioned investigators, are considerably higher than the values published by Lyngsoe (see tables 5 and 6)

Lyngsoe (154) found a rise in SILA in juvenile diabetics after administration of glucose In contrast to this Daweke (66) found a fall in SILA in this type of patient after administration of glu-

cose All the juvenile diabetics in Daweke's study were given a slowly resorbable insulin 24 hours prior to the SILA investigation, but only 25 % of the patients in Lyngsoe's study received this The reason for the difference between the two investigations, therefore, may be that Daweke, in contrast to Lyngsoe, determined the exogenous insulin in serum

Using the rat diaphragm method, normal fasting SILA values are found in older non-obese diabetics (218) Seltzer & Smith (235), examining this group of patients found a difference between Tolbutamide responders and Non Tolbutamide responders If these two groups are considered as one, however, this study also finds normal SILA values in older non-obese diabetics Most investigations by means of the rat epididymal fat method show normal or low normal fasting SILA values in this type of patient (66, 67 154 155) In older non obese diabetics there is a rise in SILA following administration of glucose

In most studies with both the rat diaphragm method and the rat epididymal fat method an elevated fasting SILA level is found in older obese diabetics Nevertheless, using the same method or the ADHA rat method a few investigators have found normal SILA values in this type of patient (40 66 67 218) Eskjær Jensen & co-workers (78) however found that the SILA level in older obese diabetic patients is dependent on the patient's intake of food In the study cited high SILA values were found in obese diabetics who received a normal diet, but after a brief period of diet with reduced carbohydrate content, SILA fell to normal values in those patients A possible reason why the investigations cited gave diverging results is that some patient groups were examined during dietary treatment, whereas other groups were examined while receiving a normal diet In line with this assumption Lyngsoe's investigations (154, 155) were carried out on patients who received a normal diet, or on a mixed population of diabetic patients on diet and diabetic patients not on diet, whereas Daweke's (66, 67) and Rizzo's investigations (218) were performed in patients under dietary treatment The other publications on older obese diabetic patients lack information on the patients caloric intake during the period of investigation

Most investigators found a rise in SILA in older obese diabetic patients after administration of glucose Phear (191) however, found a fall in the SILA level

Steinke & co-workers (254), using the rat epididymal fat method, examined SILA in a great number of older diabetic patients Their study demonstrated elevated SILA values in this group, but no information is available on the weight of the patients

Immunologically active insulin in peripheral venous blood

Most studies on serum from juvenile diabetics (tables 13 and 14) show reduced or normal fasting values for im

TABLE 13

Immunologically determined insulin in the serum from non acidotic patients with diabetes mellitus

Diabetics investigated				Fasting mean value µU/ml	Mean value after the administration of glucose µU/ml	Increase after glucose	
	Age of onset	Mean value fasting blood sugar mg %	Ketonuria				
Untreated	Older	110		27(38) high normal	156(38)¹) normal	+	Yalow & Berson (1960)[308]
Treated	Juvenile	318		7(10) decreased	4(3)¹) decreased	0	Berson & Yalow (1962)[31]
Untreated		190	−	37(17) increased	58(17)¹) normal	+	Hales & Randle (1963)[114]
		318	+	25(4) normal			

¹) *60 minutes after the oral administration of glucose*
The figures in brackets indicate the number of sera investigated

munologically-determined insulin and suppressible SILA Only one study shows elevated *suppressible* SILA in these patients (85) The reason for the divergent results obtained by these experienced investigators is not known All the patient series are small, however, and the method for determination of suppressible SILA is encumbered by a very pronounced uncertainty If all the series investigated are considered under one, it seems most likely that juvenile diabetic patients have normal or low *normal values for immunologically active* insulin in serum

Neither oral nor intravenous administration of glucose was followed by an increase in this form of insulin, in any of the published investigations on immunologically active insulin in serum from juvenile diabetics

In older, non-obese diabetic patients, reduced or normal suppressible SILA, respectively, was found in the fasting state, while no rise was found after administration of glucose (table 14)

Samaan & Fraser (228) found a normal fasting suppressible *SILA* in *older* obese diabetics, whereas Lyngsoe & Lundbæk (159) found elevated values These latter investigators observed no change in immunological SILA shortly after intravenous administration of glucose, whereas Samaan & Fraser recorded a rise 120 minutes after oral administration of glucose

Several *investigators* have recorded suppressible SILA (85) and immuno-*logically-determined insulin (114, 308)* in fairly large series of older diabetics In the publications cited, *there is no information* on the patients' weight These investigators *all found elevated or high* normal fasting values, and a rise in immunologically determined insulin after oral administration of glucose The rise

TABLE 14

Suppressible and non suppressible SILA in untreated newly diagnosed non-acidotic patients with diabetes mellitus

	Diabetics investigated			Suppressible SILA μU/ml		Non-suppressible SILA μU/ml		
	Age of onset	Mean value of fasting blood sugar mg%	Ketonuria	Fasting mean value	Mean value after the administration of glucose	Fasting mean value	Mean value after the administration of glucose	
	6–35	191	+	19(5) decreased	10(5)[1])decreased	38(5) decreased	40(5)[1])decreased	Samaan & Fraser (1963)[333]
Non-obese	39–64	211	–	15(5) decreased	1(5)[1])decreased	91(5) decreased	98(5)[1])decreased	
Obese	26–67	216	–	42(9) normal	80(9)[1])normal	203(9) increased	186(9)[1])high normal	
	Juvenile	299		44(10) increased		99(10) low normal		Froesch et al (1963)[88]
	Older	165		49(18) high normal		130(18) low normal		
	7–37	245	±	75(5) high normal	90(4)[2])low normal	110(5) normal	80(4)[2])normal	Lyngsoe & Lundbæk (1965)[188]
Non-obese	42–63	180	–	43(5) normal	100(5)[2])low normal	130(5) high normal	80(5)[2])normal	
Obese	42–69	212	–	80(8) increased	101(8)[2])low normal	133(8) increased	101(8)[2])high normal	

[1]) investigated 120 minutes after the oral administration of glucose
[2]) investigated 10 minutes after the intravenous administration of glucose
The figures in brackets indicate the number of sera investigated

TABLE 15

Immunologically determined insulin and ILA in serum extracts from non acidotic patients with diabetes mellitus

Method	Diabetics investigated				Fasting mean value μU/ml	
		Age of onset	Mean value of fasting blood sugar mg*	Keton uria		
Baird & Bornstein extraction + A D A rats	Untreated do	17–25 35–63			1000(3) low normal 4900(3) high normal	Baird & Bornstein (1959)[17]
do		Juvenile			0(7) decreased	Bornstein & Hyde (1959)[42]
		Older			(14) normal	
Baird & Bornstein extraction + rat diaphragm	Untreated non obese	16–63	390	–	(10)*)	Taylor (1963)[262]
Baird & Bornstein extraction + Arquilla & Stavitsky	Treated non-obese	Older	275	±	4500(4) decreased	Rodari et al (1961)[226]
	Treated obese	Older	165	–	18900(10) high normal	
Baird & Bornstein extraction + Arquilla & Stavitsky	Untreated obese Untreated	35–82 25–66	180 300	– +	19840(13) high normal 7410(6) decreased	Rizzo et al (1961)[219]
Resin extraction + rat diaphragm	Untreated		220		320(6) normal	Anthoniades et al (1961 • 1962[13])

*) indicates that ILA has been found
The figures in brackets indicate the number of sera investigated

after the administration of glucose occurred later than in normal subjects, and reached a higher level This result is not incompatible with the investigations just cited, performed on older obese diabetic patients nor with investigations elucidating immunologically active serum insulin in older non-obese diabetics (table 14) All the studies on older diabetics are thus in accordance with the assumption that the immunologically active serum insulin in these patients increases after administration of glucose but that this rise occurs more slowly than in normal subjects

Immunologically inactive insulin in peripheral venous blood

As previously discussed (see page 48), it is probable that a measure of the amount of immunologically inactive insulin in serum can be obtained by four different methods 1) determination of non-suppressible SILA by the rat epididymal fat method, 2) determination of ILA in warm dialyzed serum protein fractions, 3) resin treatment of serum and determination of ILA in the resin eluate, and 4) extraction of serum by Baird & Bornstein s technique. All these methods appear to have been used for examining the insulin content in serum from diabetics (tables 14, 15, and 16)

The distribution of ILA in electrophoretically separated serum protein fractions from patients with diabetes mellitus has been studied in two investigations Taylor (262) examined 6 patients with untreated diabetes mellitus and ketonuria On separation of serum on treated cellulose acetate and determination of ILA by the rat diaphragm method, he found ILA in albumin in 4 patients, and ILA in β-γ-globulin in 3 patients The examination of β-γ globulin was incomplete in two patients, and in one patient, no ILA was found in any of the fractions examined In one patient, ILA was found

TABLE 16

ILA in warm-dialyzed A and B fraction from untreated newly diagnosed non-acidotic patients with diabetes mellitus Lyngsøe & Lundbæk (1965)[148]

Age	Mean value of fasting blood sugar mg	Ketonuria	A-fraction μU/ml		B-fraction μU/ml	
			Mean fasting value	Mean value 10 min after i.v. glucose	Mean fasting value	Mean value 10 min. after i.v. glucose
7 37	245	±	2840(5) high normal	2173(3) high normal	4192(5) high normal	3833(3) high normal
42 63 non-obese	180	–	1060(5) normal	820(5) normal	1725(5) normal	1480(3) normal
42–69 obese	212	–	1500(8) normal	1576(7) normal	3933(8) increased	[illegible]670(7) increased

The figures in brackets indicate the number of sera investigated

in α globulin, in which ILA cannot be demonstrated in normal subjects by the technique employed Taylor concludes, however, that the qualitative distribution of ILA in diabetic patients does not differ from the distribution found in normal subjects

Lyngsoe & Lundbæk (159) examined electrophoretically separated serum protein fractions from 2 juvenile, 2 older non-obese and 2 older obese untreated diabetic patients, without finding any abnormal distribution of ILA in these patients

Although these investigations are too limited to exclude the possibility that abnormal fractions of immunologically inactive insulin may be present in a few diabetics, this does not seem to be the case in the great majority of patients

Quantitative examinations of immunologically inactive insulin in juvenile diabetics have given very diverging results Thus, reduced or normal values have been found for non-suppressible SILA (table 14), increased ILA has been found in both the A and the B fractions (table 16), and no or low ILA has been found in serum extracts prepared by Baird & Bornstein's technique (table 15) It is not known why ILA cannot be demonstrated in the majority of juvenile diabetics by the last-mentioned technique, but in view of the fact that by other methods, immunologically inactive insulin could be demonstrated in serum from untreated juvenile diabetics, it must be considered less likely that the examinations by Baird & Bornstein's technique give a correct measure of the amount of immunologically inactive insulin in these patients A considerably smaller fraction of the immunologically inactive ILA is determined by measurements of non-suppressible SILA than by examination of ILA in warm dialyzed A and B fractions, and it must be considered likely, therefore, that the latter technique gives the most correct measure of immunologically inactive insulin It may thus be assumed that juvenile diabetics have an increased amount of immunologically inactive insulin, corresponding to both the A and the B fractions

In older non-obese diabetics, low or normal values for non-suppressible SILA are found (table 14), as well as low values for immunologically-determined insulin in serum extracted by Baird & Bornstein's method (table 15), whereas ILA in warm dialyzed serum protein fractions is of the same order of magnitude as in normal subjects (table 16) These investigations all agree with the assumption that serum from older, non-obese diabetic patients contains reduced or normal quantities of immunologically inactive insulin

Elevated or high normal values for non-suppressible SILA were demonstrated in older obese diabetics (table 14) High normal values for immunologically-determined insulin were found on extraction by Baird & Bornstein's method (table 15), and an increase in ILA in the B fraction was found in a single investigation (table 16) These investigations thus seem to indicate that obese older diabetics have increased amounts

of immunologically inactive insulin in serum, and it is likely that this is an expression of an increase in the immunologically inactive insulin in the B fraction

Anthoniades & co-workers (9, 12), examining a number of diabetics whose characteristics were not specified, showed that these patients have normal fasting amounts of 'complex insulin' (i e ILA which can be eluted from resin) While these investigators could demonstrate a fall in complex insulin after intravenous glucose administration in normal subjects, they did not find a corresponding fall in patients with diabetes mellitus This result which is of considerable interest, has not yet been confirmed by other investigators

SERUM INSULIN IN PATIENTS IN DIABETIC ACIDOSIS

Low SILA values are reported when diluted serum from patients with diabetic acidosis is examined by the rat diaphragm method In a single study (16) in which the serum dilution is not stated no SILA was found at all Normal SILA was found in both diluted and undiluted serum by the rat epididymal fat method (table 17)

Normal values were found on examining ILA in serum extracts prepared by Baird & Bornstein's method and on determining non suppressible SILA This suggests that serum from patients in diabetic acidosis contains normal quantities of immunologically inactive insulin

It is not possible to decide whether serum from acidotic diabetics contains normal or reduced amounts of immunologically active insulin, as all the investigations elucidating the occurrence of this were made by the biological methods In spite of the fact that diluted serum has been used in these studies, it is difficult to exclude the possibility that the serum insulin antagonist present in undiluted serum from acidotic diabetics but which apparently disappears after dilution (see page 44), may inhibit the action of the excessively small amount of immunologically active insulin present in diluted serum This assumption is in agreement with investigations on suppressible SILA made by Froesch & co-workers (85) These investigators were thus unable to demonstrate suppressible SILA in serum from acidotic diabetics, in spite of finding elevated values in both juvenile and older non acidotic diabetics Froesch & co-workers investigation may of course be interpreted as a consequence of a changed insulin metabolism in patients in diabetic acidosis, but so far, no other studies have suggested such a changed insulin metabolism in this condition It may therefore be reasonable to assume that those changes which take place in the properties of the serum insulin in diabetics who develop acidosis, are due to the presence of a special serum insulin antagonist

DISCUSSION

As will appear from the preceding discussion attempts to determine insulin

in α_2-globulin, in which ILA cannot be demonstrated in normal subjects by the technique employed Taylor concludes, however, that the qualitative distribution of ILA in diabetic patients does not differ from the distribution found in normal subjects

Lyngsøe & Lundbæk (159) examined electrophoretically separated serum protein fractions from 2 juvenile, 2 older non-obese and 2 older obese untreated diabetic patients, without finding any abnormal distribution of ILA in these patients

Although these investigations are too limited to exclude the possibility that abnormal fractions of immunologically inactive insulin may be present in a few diabetics, this does not seem to be the case in the great majority of patients

Quantitative examinations of immunologically inactive insulin in juvenile diabetics have given very diverging results Thus, reduced or normal values have been found for non-suppressible SILA (table 14), increased ILA has been found in both the A and the B fractions (table 16), and no or low ILA has been found in serum extracts prepared by Baird & Bornstein's technique (table 15) It is not known why ILA cannot be demonstrated in the majority of juvenile diabetics by the last-mentioned technique, but in view of the fact that by other methods, immunologically inactive insulin could be demonstrated in serum from untreated juvenile diabetics, it must be considered less likely that the examinations by Baird & Bornstein's technique give a correct measure of the amount of immunologically inactive insulin in these patients A considerably smaller fraction of the immunologically inactive ILA is determined by measurements of non-suppressible SILA than by examination of ILA in warm dialyzed A and B fractions, and it must be considered likely, therefore, that the latter technique gives the most correct measure of immunologically inactive insulin It may thus be assumed that juvenile diabetics have an increased amount of immunologically inactive insulin, corresponding to both the A and the B fractions

In older non-obese diabetics, low or normal values for non-suppressible SILA are found (table 14), as well as low values for immunologically-determined insulin in serum extracted by Baird & Bornstein's method (table 15), whereas ILA in warm dialyzed serum protein fractions is of the same order of magnitude as in normal subjects (table 16) These investigations all agree with the assumption that serum from older, non-obese diabetic patients contains reduced or normal quantities of immunologically inactive insulin

Elevated or high normal values for non-suppressible SILA were demonstrated in older obese diabetics table 14) High normal values for immunologically-determined insulin were found on extraction by Baird & Bornstein's method (table 15), and an increase in ILA in the B fraction was found in a single investigation (table 16) These investigations thus seem to indicate that obese older diabetics have increased amounts

fasting blood sugar values in the patients examined As the pancreatic production of the immunologically active insulin (determined by means of the rat diaphragm method) is presumed to be proportional to the blood sugar level (170), it may be anticipated that increased values for immunologically active insulin in peripheral venous blood will correspond to increased fasting blood sugar This assumption is supported by Seltzer's (233) observation, that continuous intravenous perfusion of glucose into normal subjects results in a considerable increase in SILA (the rat diaphragm method) in peripheral venous blood in spite of a very moderate rise in the blood sugar values The demonstration of normal values of immunologically active insulin in diabetics with considerably increased fasting blood sugar must thus mean that relative to the increased fasting blood sugar, these values of insulin are reduced

Determination of ILA in the A and B fractions from juvenile diabetics showed elevated values in both fractions (see page 74) This agrees with the finding of Steinke & co-workers that this type of diabetic has elevated SILA values, determined by the modification of the rat epididymal fat method used by these investigators see page 68)

Thus the investigations made so far seem to indicate that in peripheral venous blood juvenile diabetics have relatively reduced values of immunologically active insulin but elevated values of immunologically inactive insulin both in the A and in the B fraction Knowledge of the insulin metabolism in the normal and diabetic organism, however, is very limited The only investigations available in diabetics elucidate the two forms of insulin in the portal vein of two patients whose characteristic data were not further specified (11) Our present knowledge of the serum insulin in peripheral venous blood from diabetics thus does not permit us to determine the locus of the defect in the insulin metabolism of these patients It must be considered established, however, that juvenile diabetics have an insulin production, in spite of the fact that no insulin has been demonstrated in an acid alcohol extract of pancreas from these patients (206, 301) After glucose administration, however there is no rise in immunologically active insulin in peripheral venous blood, so it must be assumed either that the pancreas is unable to increase the production of this type of insulin, or that the immunologically active insulin is used up or is transformed into immunologically inactive insulin peripheral to the pancreas The fact that peripheral venous serum in juvenile diabetics contains an increased amount of immunologically inactive insulin might perhaps tell in favour of the last mentioned hypothesis, this is also compatible with the fact that there is an increase in SILA (determined by the rat epididymal fat method) after ingestion of glucose by juvenile diabetics (see page 68) as this rise may be due to immunologically inactive insulin

What part the abnormality of insulin metabolism in juvenile diabetics plays

TABLE 17

Serum insulin in patients in diabetic acidosis

Method	Diabetics investigated	Total CO_2 meq/l	Dilution	Mean fasting values $\mu U/ml$	
Rat diaphragm	Diabetic coma		1/6	(4) decreased	Groen et al (1952)[104]
do	Diabetic coma and precoma	<15	1/10	106(11) decreased	Willebrands (1960)[233]
do	Diabetic coma			0(7) decreased	Baird & Bornstein (1957)[14]
Baird & Bornstein extraction + Rat diaphragm	do			(7) normal	do
Rat epididymal fat	Diabetic coma		1/10	(8) low normal	Beigelman (1959)[23]
do		<15	undil 1/5	95(5) normal 310(5) high normal	Lyngsoe (1962)[166]
do	Diabetic coma		1/5	Suppressible SILA 0(7) low normal Non suppress SILA 186(7) normal	Froesch et al (1963)[84]

The figures in brackets indicate the number of sera investigated

in peripheral venous serum from patients with diabetes mellitus have shown so far that insulin is present in serum from all types of diabetics This insulin, however, differs in several respects from insulin found in normal subjects It has further been shown that the abnormalities demonstrated in the serum insulin are probably different in the different types of diabetic patients

Juvenile diabetics

Examining juvenile diabetics, reduced or normal values were found for immunologically-determined insulin (see page 70), for SILA in diluted serum determined by the rat diaphragm method (see page 68), and for suppressible SILA (see page 70) In contrast to normal subjects, no increase in suppressible SILA or immunologically determined insulin was found in these patients after oral and intravenous administration of glucose (see page 70) These findings show that peripheral venous serum from juvenile diabetics contains immunologically active insulin, and that the activity of this type of insulin is not increased by a rise in the blood sugar This is in agreement with the observations that there was no increase in immunologically active insulin in spite of considerably increased

active insulin, triggered-off by elevated blood sugar, is less in the older non-obese diabetics This assumption is compatible with the fact that after glucose administration, the rise in immunologically active serum insulin is slower in these patients than in normal subjects

Older obese diabetics

When peripheral venous blood is examined from fasting older diabetics most studies by the rat diaphragm method find elevated SILA levels (see page 69), and in one of two investigations an increased suppressible SILA was found in patients who had not been on a diet (see page 70) Examining older obese diabetics by the rat epididymal fat method, it was found that these patients had elevated fasting SILA values when on normal diet, but that SILA and fasting blood sugar fell when they were on a diet with restricted carbohydrate content (see page 69) Correspondingly studies by the rat diaphragm method showed normal fasting SILA values in older obese diabetics on a diet (see page 69) These findings seem to indicate that untreated patients with this type of diabetes mellitus have elevated immunologically active insulin in the peripheral venous blood, and that the level becomes normalized during treatment with restricted carbohydrate intake

Some time after ingestion of glucose, a rise in SILA is found (rat diaphragm method) in peripheral venous blood in older obese diabetics (see page 69), just as there is a rise in suppressible SILA (see page 70) Shortly after intravenous administration of glucose, however, no change is found in suppressible SILA (see page 70) These findings are in agreement with the assumption that after glucose administration, the amount of immunologically active insulin in peripheral venous blood increases in older obese diabetics, but that this rise occurs more slowly in these patients than in normal subjects

Studies on the occurrence of immunologically inactive insulin in peripheral venous blood from older obese diabetics all appear to indicate the presence of an increased concentration of this type of insulin in such patients, presumably localized to the B fraction (see page 74) It is not clear whether a fall in the immunologically inactive insulin occurs in this type of diabetic during dietary treatment Thus, when serum from obese diabetics under dietary treatment was extracted by means of Baird & Bornstein's method, elevated values for immunologically determined insulin were found (see page 72), but when serum from such patients was examined by the rat epididymal fat method, no increase in SILA was observed on dilution of the serum (78) If the "dilution effect" depends on an activation of immunologically inactive insulin (see page 51) this last observation will be compatible with a decreased level for this type of insulin, whereas the first investigation reported suggests the opposite

On the basis of studies on serum insulin in peripheral venous blood from

for the insulin antagonism demonstrated in serum from such patients, is unknown (273) The serum insulin antagonism could be just as well primary as secondary, i e a result of the abnormal insulin metabolism It is also possible that both abnormalities are caused by the same diabetogenic factor in juvenile patients with diabetes mellitus

Older, non-obese diabetics

In the great majority of studies on peripheral venous blood from non obese older diabetics, normal or low normal fasting values were found for immunologically-determined insulin, suppressible SILA (see page 70), and SILA determined by the rat diaphragm method (see page 69) As the patients examined showed an elevated fasting blood sugar, these studies suggest that older non-obese diabetics have a relatively reduced level of immunologically active insulin in peripheral venous blood Using the rat diaphragm method, a rise in SILA was found in these patients after oral glucose (see page 69), presumably indicating an increase in immunologically active insulin Admittedly, this does not fit in with the finding of unchanged suppressible SILA in older non obese diabetics after ingestion of glucose (see page 70) However, considering that this result was obtained in a study including only 5 patients, whereas the investigation of SILA by the rat diaphragm method comprised a considerably more extensive patient material, the result of the latter study must be regarded as the more likely In agreement with this assumption, several of the investigations using the rat epididymal fat method have demonstrated a rise in SILA some time after administration of glucose in older non-obese diabetics (see page 69), since this rise may well be due to immunologically active insulin When suppressible SILA was determined shortly after intravenous administration of glucose, however, unchanged values were found (see page 70)

Investigations of the changes in immunologically active insulin after glucose administration in older non-obese diabetics, agree with the assumption that the activity of this type of insulin increases after glucose administration, but that the rise occurs more slowly than in normal subjects In agreement with this, immunologically-determined insulin showed a slower rise in a great number of older, untreated diabetics (whose further data were not provided), than in normal subjects (see page 70)

The studies which have so far aimed at elucidating the characteristics of serum insulin in older non-obese diabetics, appear to be unanimous in showing an insulin production in these patients In peripheral venous blood, however, relatively reduced fasting values for immunologically active insulin seem to be found together with normal or low normal values for immunologically inactive insulin, it appears likely, therefore that the insulin metabolism in these patients is similar to that of normal subjects but that the production of immunologically

kl				Eskjær Jensen et al (1963)[6]
	60)	700(12) normal	–	Phear (1962)[181]
	60[1])	750 (6) normal	–	
	120[1]	375(6) decreased	–	
	60[1])	540(7) low normal	–	Dawcke (1963[46] 1963)[47]
	60[1])	380(5) decreased	–	
	60[1])	360(12) decreased	+	
	60[1])	454(7) decreased	+	

older obese diabetics, it must be concluded that an insulin production is present in these patients, and since the level of immunologically active insulin varies with changes in the blood sugar, it seems reasonable to assume that the insulin production responds to changes in the blood sugar In dietary-treated diabetics of this type, however, when normal SILA values (presumably reflecting a normal level of immunologically active insulin) are found together with elevated fasting blood sugar, this may be taken as expressing an "abnormality of regulation" in the insulin production Our knowledge of insulin metabolism in normal subjects is too inadequate, however, to decide whether this hypothesis is compatible with the findings of a higher level of immunologically inactive insulin in the B fraction, and, following glucose administration, of a slower rise in immunologically active insulin in peripheral venous blood, than in normal subjects

The hypothesis presented, however, does not explain why increased fasting blood sugar is found in untreated older obese diabetics, in spite of an elevated fasting level of immunologically active insulin and a presumably increased production of insulin Nevertheless, investigations suggest that these patients have an increased production of growth hormone before they commence treatment (75, 78), and this may give rise to a decreased insulin sensitivity in the musculature (88) It is reasonable, therefore, to assume that an increased production of growth hormone may cause an increase in fasting blood sugar, in spite of a presumably increased production of insulin Whether the cause of the diabetic condition in the older obese patients is an increased production of growth hormone, or whether this increase is secondary to the diabetes, is nevertheless not clear This problem might be solved by studies on the production of growth hormone and insulin in the prediabetic condition in older obese patients, as well as on the changes in the amount of the different types of insulin in peripheral venous serum from older obese diabetics under treatment with diet and growth hormone

CHAPTER 6

INSULIN CONTENT OF BLOOD OF PATIENTS OBESITY AND PATIENTS WITH ACROMEGALY

NSULIN IN NON DIABETIC IENTS WITH OBESITY

n in ulin in obese patients es ellitus ha been investi reat n umber f tudies see rre ponding, n vestigations en t e e patients are few Table 18 h ws that normal

er high normal fasting SILA values are found in peripheral venous blood from such patients (the rat epididymal fat method) as well as high normal fasting values for immunologically determined insulin after extraction of the serum by acid alcohol In one study 223 different levels were found for immunologically determined insulin in

TABLE 18

S n u n d abe ic pa ient u h ob y

			M ar h	60 m n af er adm n st on f gl ucose	
ar + ak	ased		150 000 f	ncreased	Rodari et al (1960)[139]
k	o al		20 4	normal	
t n	gl o al		800 10	h gh norm al	Phear (1962
d	al				Lyngsoe (1962
	n ased				Rabinow iz & Z erler 1962)
ur	6 n a		140 10	ncreased	Karam et al. (1963)

w ns
s w ns
f a pla a
1 a fn n er d glucose
u xi a 1 num ber of sera n est gated

THE INSULIN CONTENT OF BLOOD OF PATIENTS WITH OBESITY AND PATIENTS WITH ACROMEGALY

SERUM INSULIN IN NON DIABETIC PATIENTS WITH OBESITY

While serum insulin in obese patients with diabetes mellitus has been investigated in a great number of studies (see page 79) corresponding investigations on non diabetic obese patients are few in number Table 18 shows that normal or high normal fasting SILA values are found in peripheral venous blood from such patients (the rat epididymal fat method) as well as high normal fasting values for immunologically determined insulin after extraction of the serum by acid alcohol In one study (293) different levels were found for immunologically determined insulin in

TABLE 18

Serum insulin in non-diabetic patients with obesity

Method	Mean fasting value μU/ml		Mean value 60 min after oral administration of glucose		
[illegible] ethanol extraction [illegible] qu lla & S a sky	34 500 (6)	increased	150 000 (6)	increased	Rodari et al (1960)
	9 200 (5)[a]	normal	56 200 (4)[b]	normal	
Rat epididymal fat method	625 (10)	high normal	800 (10)	high normal	Phear (1962)
	87 (7)	normal			Lyngsoe (1962)
Yalow & Berson's immunological method	31 (6)	increased			Rabinowitz & Zierler (1962)
[illegible] extraction method with immunological method	60 (10)	high normal	140 (10)	increased	Karam et al (1963)

[illegible] patients with obesity

Untreated patients with obesity

Investigated in peripheral arterial plasma

Investigated after intravenously administered glucose

The figures in brackets indicate the number of sera investigated

tients with and without diabetic metabolic anomaly

These investigations on SILA in patients with acromegaly make it reasonable to assume that an increased production of growth hormone may cause an increase in SILA This assumption is in agreement with studies in which an elevated fasting SILA was found after administration of growth hormone to normal subjects (Zahnd et al 306) and to experimental animals (205) Stein & co-workers (251), however, have not been able to confirm these findings by administration of growth hormone to normal subjects although these investigators used a technique which is almost identical to that of Zahnd with the exception that the growth hormone was administered over a longer period

It is of considerable interest that Zahnd & co-workers (306) found a rise in SILA 4 hours after intravenous administration of growth hormone without the occurrence of a corresponding fall in the blood sugar Assuming that the rise in SILA reflects an increased production of insulin, a fall in the blood sugar might have been anticipated concurrently with the rise in SILA As this does not seem to be the case, it is reasonable to assume that the growth hormone has caused a decreased sensitivity to insulin in the musculature (88), and to consider the increased insulin production as being a compensatory reaction to this An interesting observation in this connection is that after long term administration of growth hormone to experimental subjects, Stein & co-workers (251) found that glucose ingestion was followed by a much higher rise in SILA level in these subjects than in normal subjects who had not been treated with growth hormone The assumption that an increased production of insulin following administration of growth hormone expresses an indirect effect, gains support from a recently published study (104) This shows that isolated perfused rat pancreas does not increase its production of immunologically-determined insulin after stimulation with growth hormone

serum extracts from obese patients of two differing types, elevated fasting values in "gynoid" and normal fasting values in "android" obese patients. The investigations mentioned deserve attention in spite of the uncertainty of the objective basis for this distribution of obesity into two types, and in spite of the limited number of patients studied.

In peripheral venous blood from obese patients, glucose administration was followed by a rise in SILA and in immunologically-determined insulin in serum extracts, to values higher than those determined in serum from normal subjects (table 18).

A single investigation of immunologically-determined insulin in arterial blood from obese patients showed elevated fasting values (table 18).

The investigations cited, in which serum insulin is studied in non diabetic patients with obesity, do not permit any precise description of the abnormalities which are present in the insulin metabolism of these patients. In view of the fact that both elevated SILA and immunologically-determined insulin may be found after glucose administration, it nevertheless seems reasonable to assume that non-diabetic patients with obesity will respond to glucose administration by an increased production of insulin. It is probable that the increased fasting level of immunologically-determined insulin in peripheral arterial blood is due to the fact that the insulin production is also increased during the fasting state. There are no investigations, however, elucidating the type of stimuli which set off the increased insulin production, just as it is not known whether the increased insulin production is secondary or primary to the development of obesity.

SERUM INSULIN IN PATIENTS WITH ACROMEGALY

The most comprehensive investigations elucidating serum insulin in acromegalic patients have been made with the rat diaphragm method. Randle (200, 204) found definitely elevated SILA values in patients with acromegaly, while Wright (304) found increased fasting SILA in acromegalic men, but not in acromegalic women.

Examination of 3 acromegalic patients by means of the rat epididymal fat method showed high normal values (155), whereas Yalow & Berson (308) found a normal fasting level of immunologically-determined insulin in two patients. Investigations of immunologically-determined insuling in acid alkohol extracts from serum showed elevated values in 3 acromegalic patients (130).

Wright (304) compared the SILA level in acromegalic patients with varying "activity" of the disease, but found no relationship. This seems a reasonable result in view of the difficulties of deciding whether a patient is suffering from active or inactive acromegaly. On the other hand, it is astonishing that none of the more comprehensive investigations on acromegaly have made any comparisons between SILA levels in pa-

tients with and without diabetic metabolic anomaly

These investigations on SILA in patients with acromegaly make it reasonable to assume that an increased production of growth hormone may cause an increase in SILA. This assumption is in agreement with studies in which an elevated fasting SILA was found after administration of growth hormone to normal subjects (Zahnd et al 306) and to experimental animals (205). Stein & co-workers (251), however, have not been able to confirm these findings by administration of growth hormone to normal subjects, although these investigators used a technique which is almost identical to that of Zahnd, with the exception that the growth hormone was administered over a longer period.

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The investigations cited, in which serum insulin is studied in non diabetic patients with obesity, do not permit any precise description of the abnormalities which are present in the insulin metabolism of these patients In view of the fact that both elevated SILA and immunologically-determined insulin may be found after glucose administration, it nevertheless seems reasonable to assume that non-diabetic patients with obesity will respond to glucose administration by an increased production of insulin It is probable that the increased fasting level of immunologically-determined insulin in peripheral arterial blood is due to the fact that the insulin production is also increased during the fasting state There are no investigations however, elucidating the type of stimuli which set off the increased insulin production, just as it is not known whether the increased insulin production is secondary or primary to the development of obesity

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mellitus exhibit different abnormalities of serum insulin The factors responsible for these abnormalities are not known, and at present no more can be concluded than that all diabetics must be assumed to have an abnormal insulin production and/or metabolism Whether these abnormalities in themselves cause diabetes mellitus, or whether they are secondary to other conditions which determine the diabetic state, is not known It is an obvious point in this connection that an abnormal production of serum insulin antagonists in the prediabetic and diabetic states may be assumed to involve abnormalities in the serum insulin Our present knowledge, however does not permit us to decide which of the antagonists described is responsible and it certainly does not permit us to formulate any conclusions as to how the mechanism functions It should also be stressed that the whole question of the type and number of serum insulin antagonists is so unsettled that it is only with the greatest caution that any importance dare be ascribed to them in the pathogenesis of diabetes One of the most significant tasks for the future, therefore, must be to elucidate the relationship between the serum insulin antagonists so far described With this achieved, it will be reasonable to suppose that studies on the occurrence of serum insulin antagonism, on the different types of serum insulin in "prediabetics" and on different types of experimental diabetes, will increase our knowledge of the pathogenesis of diabetes mellitus

FINAL COMMENTS

Our knowledge with regard to serum insulin has been considerably extended during recent years, but at the same time our conception of the properties of the insulin in serum has become more complex It must now be accepted as a fact that untreated serum contains considerable amounts of insulin in a form which is biologically inactive *in vitro*, so that more than one type of insulin is present in serum As a result of recent studies showing that commercially purified pancreatic insulin may be antigenic to the animal species from which it has been prepared, the question has been raised whether the insulin molecule in serum differs from the insulin molecule in the pancreas It is not clear whether this is in some way a consequence of the same phenomenon which is responsible for serum insulin occurring in a variety of forms, or whether it is due to the fact that pancreatic insulin is altered during the extraction procedure Studies to determine whether the serum insulin is associated with the plasma proteins, and if so, how, may possibly provide a reply to this question, and at the same time may explain what happens when serum is treated by "warm dialysis" Closely related to these problems is the question of the relationship between the values obtained by the different methods for determining immunologically inactive insulin It is possible that the terms "non suppressible SILA", "complex insulin" and "ILA of A and B fraction", all represent different insulin fractions, but it is also conceivable that one and the same fraction can be determined by a variety of methods

As has been discussed previously, our knowledge of the physiological significance of the different types of insulin is very limited It is probable that immunologically active insulin is biologically active *in vivo*, but whether immunologically inactive insulin has an *in vivo* effect, perhaps only in certain tissues, and whether there is a difference in this respect between the A and the B fractions, is not known Closely related to this problem are the questions of the site of formation of the different fractions of immunologically inactive insulin, of where they are consumed, and of whether the production and the consumption vary with the metabolic state of the subject.

The present investigations on serum insulin in diabetics have furnished us with the very significant information that different clinical types of diabetes

described The mean concentration of immunologically active insulin in normal fasting serum amounts to between 10 and 50 uU/ml, the concentration increasing following administration of glucose This type of insulin is produced in the pancreas and is partly retained in the liver, and presumably also peripherally The normal values for immunologically inactive insulin vary considerably, depending on the methods used to determine this type of insulin The sites of formation and of consumption of immunologically inactive insulin are not known

Chapter 5

Studies on the serum insulin in patients with diabetes mellitus have shown that insulin is present in the serum in all types of diabetics but that it differs in a number of ways from the insulin in normal subjects Different clinical types of diabetics show different kinds of serum insulin abnormality Relatively low values of immunologically active insulin are found in peripheral venous serum from fasting juvenile diabetics No rise is observed in this type of insulin following administration of glucose Juvenile diabetics have presumably an increased amount of immunologically inactive insulin in serum, corresponding to both the A fraction and the B fraction

Peripheral venous serum from older non-obese diabetics shows relatively low fasting values for immunologically active insulin The rise in concentration of this type of insulin after glucose administration is slower in these patients than in normal subjects These diabetics show normal amounts of immunologically inactive insulin, presumably corresponding to both the A and the B fraction

Elevated values of immunologically active insulin are found in peripheral venous blood from untreated, fasting, older obese diabetics After glucose administration the rise in this insulin is slower in these patients than in normal subjects This type of diabetic shows an increased concentration of immunologically inactive insulin, presumably localized to the B fraction

Chapter 6

Studies on patients with obesity and patients with acromegaly indicate that these two diseases are accompanied by an increased insulin production The mechanisms triggering off this production in patients with obesity are unknown Growth hormone, however, is known to be insulinotropic, but whether this is a direct or an indirect effect is uncertain

SUMMARY

Chapter 1

The methods for determining insulin in small concentrations are discussed, with a view to their applicability to determining insulin in serum. None of these methods are specific for the biologically active insulin molecule. In normal serum, however, the rat diaphragm method, the rat epididymal fat method and the immunological methods based on Yalow & Berson's principle will presumably determine insulin alone.

Chapter 2

Studies are discussed which throw light on the occurrence of different types of serum insulin. With this background, the theory is presented that two types of insulin exist in serum. One type—the immunologically active type—can be bound to anti-insulin, while the second type—the immunologically inactive type—cannot be so bound. The immunologically inactive type of insulin presumably occurs in at least two fractions. One fraction is localized to albumin α_1-globulin, the other fraction to β_1-globulin.

Chapter 3

Serum insulin antagonists have been demonstrated in normal subjects, diabetics, and animals with experimental diabetes. All the antagonists described can be demonstrated by the rat diaphragm method. For most of the antagonists, it is not reported whether they are active in relation to rat epididymal fat, but the synalbumin antagonist described by Vallance-Owen cannot be demonstrated by this method. On the other hand, the method determines antagonism in serum from patients in diabetic acidosis. Several of the antagonists described in man and experimental animals are dependent on an intact hypophyseal and adrenocortical function, but it is not known whether any of the antagonists described in experimental animals correspond to those known in man. Nor is it clear whether some of the insulin antagonists described in man are in fact identical with each other.

Chapter 4

The studies on serum insulin in normal subjects and experimental animals are

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RESUME

Kapitel 1

Methoderne til registrering af insulin i små concentrationer gennemgås med henblik på deres anvendelighed til bestemmelse af insulin i serum Ingen af disse methoder er specifikke for det biologisk aktive insulinmolekyle, men i normalt serum må rottediafragmamethoden, rotteepididymisfedtmethoden og de immunologiske methoder baseret på Yalow & Bersons princip formodes kun at bestemme insulin

Kapitel 2

Undersøgelser der belyser forekomsten af forskellige former for seruminsulin diskuteres På baggrund heraf fremsættes den teori, at insulin forekommer i to former i serum, i den ene form – den immunologisk aktive – kan det bindes til antiinsulin, i den anden form – den immunologisk inaktive – kan det ikke bindes Den immunologisk inaktive form for insulin må antages at forekomme i mindst to fraktioner, den ene lokaliseret til albumin α_1-globulin den anden til β-γ-globulin

Kapitel 3

Seruminsulin antagonister er påvist hos normalpersoner, diabetikere og eksperimentelt diabetiske forsøgsdyr Samtlige beskrevne antagonister er påviselige med rottediafragmamethoden For de fleste antagonisters vedkommende er det uoplyst, om de er aktive overfor rotteepididymisfedt, men den af Vallance Owen beskrevne synalbuminantagonist kan ikke påvises med denne methode, der derimod registrerer antagonisme i serum fra patienter i diabetisk acidose Flere af de antagonister, der er beskrevne hos mennesker og forsøgsdyr, er afhængige af intakt hypofyse og binyrebarkfunktion Men om nogle af de antagonister, der er beskrevne hos forsøgsdyr svarer til de, der kendes hos mennesker er ukendt, og det er ligeledes uklart om nogle af de hos mennesker beskrevne insulinantagonister i virkeligheden er identiske

Kapitel 4

Undersøgelser af seruminsulin hos normalpersoner og forsøgsdyr omtales I normalt fasteserum er den gennemsnitlige koncentration af immunologisk aktivt insulin mellem 10 og 50 μU/ml efter glukose stiger koncentrationen Denne form for insulin produceres i pancreas og retineres partielt i leveren og formodentlig også i periferien Normalværdierne for immunologisk aktivt insulin

varierer betydeligt, afhængigt af hvilke methoder der anvendes til registrering af denne form for insulin Det er ukendt hvor det immunologisk inaktive insulin dannes og forbruges

Kapitel 5

Arbejder der belyser seruminsulinets forhold hos patienter med diabetes mellitus har vist, at der hos alle typer diabetikere findes insulin i serum, men at dette på flere måder forholder sig anderledes end hos normalpersoner Seruminsulinabnormiteterne er af forskellig art hos diabetikere af forskellig klinisk type. I perifert venøst serum fra fastende juvenile diabetikere findes relativt nedsatte værdier for immunologisk aktivt insulin, efter glukoseindgift ses ingen stigning i denne form for insulin De juvenile diabetikere har antagelig en øget mængde immunologisk inaktivt insulin i serum såvel svarende til A som til B fraktionen I perifert venøst serum fra ældre ikke adipøse diabetikere findes relativt nedsatte fasteværdier for immunologisk aktivt insulin Efter glukoseindgift stiger koncentrationen af denne form for insulin langsommere end hos normalpersoner Hos disse diabetikere findes normale mængder immunologisk inaktivt insulin formodentlig såvel svarende til A som til B fraktionen I perifert veneblod fra ubehandlede, fastende, ældre adipøse diabetikere findes forhøjede værdier for immunologisk aktivt insulin, efter glukoseindgift stiger dette langsommere end hos normalpersoner Hos denne type diabetikere findes en øget koncentration af immunologisk inaktivt insulin, antagelig lokaliseret til B fraktionen

Kapitel 6

Undersøgelser af patienter med adipositas og patienter med acromegali tyder på, at disse to sygdomme er ledsaget af en øget insulinproduktion Det er ukendt hvilke mekanismer, der udløser denne hos patienter med adipositas Derimod vides det, at væksthormon er insulinotropt, men om dette er en direkte eller indirekte effekt er uvist

REFERENCES

1 Anderson, E, Lindner, E & Sutton, V *Amer J Physiol 149* 350, 1947 (15)

2 *Anderson, E, Wherry, F, Bates, R W &* Cornfield, J *Proc Soc Exp Biol Med 94* 321, 1957 (14, 15)

3 Anderson, E, Wherry, F *Proc Soc Exp Biol Med 111* 446, 1962 (15)

4 Andreani, D, Negri, M, Sereno, L Greco, V & Cramarossa, L *Rass Fisiopat Clin & Ther 32* 1146, 1960 (41, 59)

5 Anthoniades, H N *Science 127* 593, 1958 (36)

6 Anthoniades H N *Endocrinology 68* 7, 1961 (37)

7 Anthoniades, H N *Diabetes 12* 365 1963 (36, 37, 40, 41)

8 Anthoniades, H N, Beigelman P M Tranquada, R B & Gundersen, K *Endocrinology 69* 46, 1961 (36)

9 Anthoniades, H N, Gundersen K & Pyle, H M *Endocrinology 69* 163, 1961 (51 52, 59, 72, 75, 81)

10 Anthoniades H N & Gundersen, K *Endocrinology 69* 36, 1961 (37)

11 Anthoniades, H N, Bougas J A & Pyle, H M *New Engl J Med 267* 218 1962 (51, 52 59, 62, 72, 77)

12 Anthoniades, H N Bougas, J A, Pyle H M Camerini Davalos R & Gundersen K *New Engl J Med 267* 953, 1962 (51, 53, 59 62 75, 81)

13 Arquilla, E R & Stavitsky, A B *J Clin Invest 35* 458 1956 (31 33)

14 Arquilla, E R Rodari, T & Crosignani, A *Arch Sci Med* (Tor) *109* 471, 1960 (64)

15 Arquilla, E R Alexander J D Ptacek, E D & Loosli E S *Diabetes 11* 412 1962 (15 16)

16 Baird, C W & Bornstein J *Lancet I* 1111, 1957 (44, 58 75 76)

17 Baird C W & Bornstein J *J Endocr 19* 74, 1959 (14 15, 16, 58, 59 72)

18 Baird J D & Farquhar, J W *Lancet I* 70, 1962 (21, 62)

19 Ball E G & Merrill, M A *Endocrinology 69* 596, 1961 (26 50 52, 53, 55)

20 Ball, E G & Knobil E *Endocrinology 72* 658, 1963 (50, 64)

21 Barlett, G R, Wick, A N & Mac Kay E M *J Biol Chem 178* 1003 1949 (23)

22 Beigelman P M *Diabetes 7* 365, 1958 (15, 37)

23 Beigelman P M *Diabetes 9* 27, 1959 (26, 76)

24 Beigelman, P M *Metabolism 9* 580, 1960 (26)

25 Beigelman P M, Goetz F C Anthoniades H N Thorn G W *Metabolism 5* 35, 1956 (14)

26 Beigelman, P M Anthoniades, H N Goetz F C Renold A E Oncley J L & Thorn G W *Metabolism 5* 44 1956 (15, 36)

27 Beigelman, P M & Onoprienko, I S *Diabetes 8* 438 1959 (37)

28 Beigelman, P M & Tranquada R E *Diabetes 11* 179 1962 (81)

29 Berson S A Yalow R S Bauman A Rotschild, M A & Newerly K *J Clin Invest 35* 170 1956 (31 41 45)

30 Berson S A & Yalow R S *Amer J Med 31* 874 1961 (27)

31 Berson S A & Yalow R S *In CIBA foundation colloquia on endocrinology* Vol 14 Immunoassay of Hormones Churchill London 1962 Pag 182–200 (32 33 34 41, 70)

32 Bethune J E Despointes R H Anthoniades H N & Nelson O H *Proc Soc Exp Biol Med 97* 69 1958 (23)

33 Bolinger R E ~ d Geld H ~ Wille

brands \ F *Metabolism 8* 39, 1959 (37)
34 Bornstein J *Austr J Exp Biol Med Sc 28* 87 1950 (15)
35 Bornstein J *Austr J Exp Biol Med Sc 28* 93 1950 (52)
36 Bornstein J *Med J Austr I* 119 1951 (42)
37 Bornstein J *J Biol Chem 205* 513 1953 (46 47)
38 Bornstein J & Trewhella P *Lancet II* 678 1950 (23)
39 Bornstein J & Trewhella P *Austr J Exp Biol Med Sc 28* 569 1950 (81)
40 Bornstein J & Lawrence R D *Brit Med J II* 1541 1951 (52 69 81)
41 Bornstein J & Park C R *J Biol Chem 205* 503 1953 (45 46)
42 Bornstein J & Hyde D Quoted by Baird C W & Bornstein J *J Endocr 19* 74 1959 (72)
43 Bornstein, J & Hyde D *Diabetes 8* 92 1960 (44)
44 Brown D H Park C R Daughaday W H & Cornblath M *J Biol Chem 197* 167 1952 (17)
45 Brugsch H & Horsters H *Arch Exper Path & Pharm 148* 295 1930 14)
46 Burrows B A *J Clin Invest 36* 393 1957 (45)
47 Burgi H Ramseier E B Froesch E R Bally P & Labhart A *Helv Med Acta 29* 527 1962 (27 28 53 55 57)
48 Burgi H Schwarz K Kopetz K & Froesch E R *Lancet II* 314 1963 (8 65
49 Cahill C F Leboeuf B & Renold A E *J Biol Chem 234* 2540 1959 25)
50 Cahill C F Leboeuf B & Flinn R E *J Biol Chem 235* 1246 1960 (29
51 Candela J L R *Ciba Found Coll Endocrinology 6* 233 1953 (24)
5 Candela J L R & Candela R R *Rep III Kongr Internat Diab Federation* Dusseldorf 1958 pag 762 763 64
53 Candela J L R *Rev Iber de Endoc 6* 171 1959 (25
54 Candela J L R & Lopez Quijada C *Med Exp 2* 40 1960 31
55 Castrillon A M Garcia Fernandez M C Candela R R & Candela J L R *Proc Soc Exp Biol Med 109* 172 1962 24 30)
56 Ceresa, F & Reyneri C *Acta Endocr 12* 85 1953 (23)
57 Clarke D W & Forbath N *Metabolism 8* 553 1959 (25)
58 Clausen J Gjedde F & Jorgensen K *Proc Soc Exp Biol Med 112* 778, 1963 (37)
59 Cohen, G & Goldenberg M *J Neurochem 2* 71 1957 (24)
60 Cohn E J Strong L E Hughes Jr W L Mulford D J Ashworth J N Melin M & Taylor H L *J Am Chem Soc 68* 459 1946 (36 42)
61 Colwell A R & Weiger R W *J Lab Clin Med 47* 844 1956 (45)
62 Correa P R & Magalhaes E *Metabolism 10* 808 1961 (25 29)
63 Cunningham N F *J Endocr 25* 35 1962 (18 19 20 21)
64 Cunningham, N F *J Endocr 25* 43 1962 (64)
65 Davidson J K Haist R E & Best C H *Diabetes 12* 448 1963 (60)
66 Daweke H *Acta Endocr 42* 437 1963 (53 55 68 69 81)
67 Daweke H *Personal communication 1963 (69 81)*
68 Debro J R Tarver H & Korner A *J Lab Clin Med 50* 728 1957 (42)
69 Ditschuneit H Chang Su Ahn Pfeiffer M & Pfeiffer E F *Klin Wschr 37* 1234 1959 (25 53)
70 Ditschuneit H Pfeiffer E F & Rossenbeck H G *Klin Wschr 39* 71 1961 (26 31)
71 Ditschuneit H Ziegler R & Pfeiffer E F *Klin Wschr 39* 426 1961 (29)
72 Doisy R J *Endocrinology 72* 273 1963 (53 64)
73 Dole V P *J Biol Chem 237* 2758 1962 (30)
74 Egdahl R H & Goldberg H *Surgery Gynec Obstr 114* 202 1962 (27 33 63 64)
75 Ehrlich R M & Randle P J *Lancet II* 230 1961 (23 80)
76 Eik Ness, K *In "Hormones in Human plasma* Little Brown & Co Boston 1960 pag 358 (23)
77 Ensink J & Vallance Owen J *Diabetes 12* 353 1963 (43)
78 Eskjær Jensen S Lundbæk, K & Lyngsøe

J *Acta Med Scand 174* 769, 1963 (68, 69, 79, 80, 81)
79 Ezrin, C & Moloney, P J *J Clin Invest 38* 1002, 1959 (45)
80 Field, J B *J Clin Invest 38* 551, 1959 (45)
81 Field, J B & Stetten de Witt Jr *Diabetes 5* 391, 1956 (44)
82 Field, J B, Tietze, F Stetten de Witt Jr *J Clin Invest 36* 1588, 1957 (44)
83 Field, J B & Rigby, B *J Endocr 19* 174, 1959 (44)
84 deFilippis, V & Iannacone, A *Lancet I* 1192, 1952 (45)
85 Froesch, E R, Burgi, H, Ramseier, E B, Bally, P & Labhart, A *J Clin Invest 42* 1816, 1963 (70, 71, 75, 76)
86 Fujita, T *J Clin Endocr 17* 512, 1957 (23)
87 Gaddum, S H *Pharmacol Rev 5* 87, 1953 (15)
88 Galbraith, H B, Ginsberg J & Paton, A *Diabetes 9* 459, 1960 (80, 84)
89 Gardiner, D C, Martin Hernandes, D & Young F G *Lancet II* 1269, 1960 (35, 36)
90 v d Geld, H, Willebrands, A F & Groen J *Bull Res Council Israel 6 E* 129, 1958 (25)
91 Gellhorn E, Feldman, J & Allen A *Endocrinology 29* 137, 1941 (14, 15)
92 Gellhorn E Feldman, J & Allen A *Endocrinology 29* 849, 1941 (16, 52, 64)
93 Gemmill, C L *Bull Johns Hopkins Hosp 66* 232, 1940 (16)
94 Gemmill, C L *Bull Johns Hopkins Hosp 68* 50, 1941 (16)
95 Gemmill, C L & Haman L jr *Bull Johns Hopkins Hosp 68* 329, 1941 (16)
96 Gjedde, F *Personal communication* 1960 (28)
97 Gjedde, F *Adv Abstracts I Int Congr Endocr* Periodica Copenhagen 1960 p 1245 (37)
98 Gjedde, F *Personal communication* 1963 (54, 55)
99 Goetz, F C, Beigelman P M & Thorn G W *Proc Soc Exp Biol Med 36* 484, 1954 (14 15)
100 Goetz F C & Greenberg B *J Lab & Clin Med 58* 819, 1961 (32)
101 Griswold, R L *J Appl Physiol 12* 117 1958 (24)
102 Grodsky, G M, Peng, C T & Forsham, P H *Arch Biochem Biophys 81* 264, 1959 (34)
103 Grodsky, G M & Forsham, P H *J Clin Invest 39* 1070, 1960 (32, 33, 58, 59)
104 Grodsky, G M, Bennet, L, Batts, A, Mc Williams, N & Vcella, N *Fed Proc 21* 202, 1962 (34, 84)
105 Groen, J, Kaminga, C E, Willebrands, A F & Blickman, J R *J Clin Invest 31* 97, 1952 (16, 18, 22, 53, 76 81)
106 Groen, J, v d Geld, H, Bolinger R E & Willebrands, A F *Diabetes 7* 272, 1958 (24, 45, 50)
107 Gundersen, K & Anthoniades H N *Proc Soc Exp Biol Med 104* 411, 1960 (36, 51)
108 Gundersen, K & Williams R H *Proc Soc Exp Biol Med 105* 390, 1960 (44)
109 Gundersen, K & Lin, B J *Tufts Folia Medica 8* 17, 1963 (50)
110 Hagen, J H *J Biol Chem 236* 1023, 1961 (29)
111 Hagen, J H, Ball E G & Cooper, O *J Biol Chem 234* 774, 1959 (25 26)
112 Hagen, J H & Ball E G *J Biol Chem 235* 1545 1960 (25 29)
113 Hales C N & Randle P J *Lancet I* 200, 1963 (32, 34, 56)
114 Hales, C N & Randle, P J *Lancet I* 790 1963 (56, 70)
115 Hales, C N & Randle P J *Biochem J 88* 137, 1963 (33, 50)
116 Hales, C N & Kennedy, G C *Biochem J 90* 620, 1964 (63, 64)
117 Hartog M & Fraser R *J Endocr 22* 101, 1961 (23)
118 Hausberger, F X, Milstein, S W & Rutman, E J *J Biol Chem 208* 431, 1954 (25)
119 Hemmingsen A Nielsen, A & Nielsen A L *Acta Med Scand Suppl 90* 105 1938 (14 15)
120 Hendley, E D Bregman E & Krahl M E *J Biol Chem 226* 459 1957 (46)
121 Hill, J B *Proc Soc Exp Biol Med 102* 75 1959 (19)
122 Holt C v Holt L v Kröner B & Kuhnau J *Science 125* 735 1957 (64)

123 Humbel R E *Experientia 15* 256, 1959 (26 28 29, 52)
124 Humbel R. E Volm K. Froesch E R & Labhart A. *Schweiz Med Wschr 89* 1217 1959 (61)
125 Havaldsen R. & Walas O *Acta Chem Scand 11* 1085 1957 (24)
126 Itzhaki S & Wertheimer, E *Endocrinology 61* 72 1957 (25)
127 Jeanrenaud B G & Renold A E *J Biol Chem 235* 2217 1960 (29)
128 Jessup D C & Wiberg C S *Diabetes 10* 201 1961 (17 18 21)
129 Jungas R L & Ball E G *J Biol Chem 235* 1894 1960 (29)
130 Karam J H Grodsky C M & Forsham P H *Diabetes 12* 197 1963 (58 59 82 83)
131 Kass M Mc Garry E & Rosenfeld I *Diabetes 4* 133 1953 (45)
132 Keen H *Diabetes 12* 406 1963 (43)
133 Kipnis D M Riddick F A Williamson J & Lacy P *J Lab & Clin Med 58* 836 1961 (46)
134 Kleeberg J Diengott D & Gottfried J *J Clin Endocr 16* 680 1956 (45)
135 Kosaka K Ide T Kuzuya, N & Kuzuya T *Endocrinology 72* 552 1963 (15)
136 Krahl M E & Park C R *J Biol Chem 174* 939 1948 (16)
137 Krahl M E & Wool I G *Abstr IVth Intern Congr Biochem Vienna* Abstr 9 4 102 1958 (16)
138 Krahl M E Tidball M E & Bregman *Proc Soc Exp Biol Med 101* 1 1959 (16)
139 Langdon R G *J Biol Chem 235* PC 15–16 1960 (30)
140 Leake N H & Burt R L *Diabetes 11* 419 1962 (26 53)
141 Leboeuf B Flinn R B & Cahill G F *Proc Soc Exp Biol Med 102* 527 1959 (25 29
142 Leboeuf B & Cahill G F *J Biol Chem 238* 41 1961 (29)
143 Leboeuf B Renold A E & Cahill G F *J Biol Chem 237* 988 1962 (29)
144 Lee H M Ellis R M & Brower W W *Proc Soc Exp Biol Med 104* 4 1960 (29)
145 Leonards J R *Fed Proc 18* 272 1959 (27)
146 Leonards, J B, Landau B R & Bartsch G *J Lab Clin Med 60* 552 1962 (25 26 27, 28)
147 Leupin, E & Verzar F *Nature* (London) *163* 836 *1949* (23)
148 Lever W F, Gurd F R. N Uroma E Brown B K, Barnes B A Schmid, K. & Schultz, E L *J Clin Invest 30* 99 1951 (35 44)
149 Lowell, F C *J Clin Invest* 23 233 *1944* (45)
150 Lowy C Blanchard C & Phear D *Lancet I* 802 1961 (43)
151 Lynn W S, Mc Leod R M & Brown R H *J Biol Chem 235* 1004, 1960 (29)
152 Lyngsoe, J *Scand J* Clin Lab Invest *13* 628, 1961 (25, 26 50 52 53 54, 55)
153 Lyngsoe J *Acta Med Scand 171* 365 1962 (28 50 51, 52 53 55 82)
154 Lyngsoe J *Acta Med Scand 172* 41 1962 (68 69, 81)
155 Lyngsoe J *Acta Med Scand 172* 601 1962 (28 43 44 45 46 50 64 68 69, 76 81 83)
156 Lyngsoe J *Acta Med Scand 174* 589, 1963 (28 37, 38)
157 Lyngsoe J *Acta Med Scand 175* 401 1964 (37 38 50 52 53 55 60 61)
158 Lyngsoe J *unpublished observation* 1964 (30 40 41)
159 Lyngsoe J & Lundbæk K. *Acta Med Scand in press* 1965 (28 55 57 60 70 71 73 79)
160 McGarry E E Fishman J Lesnoff A Simpson R Lucius A Ballantyne A & Beck J C *Acta Endocr suppl 51* 290 1960 (23)
161 Manchester K. L & Young F G *Biochem J 70* 353 1958 (16 19)
162 Manchester K L., Randle P J & Smith G H *Brit Med J I* 1028 1958 (25)
163 Manchester K L & Young F G *J Endocr 18* 381 1959 (22 23)
164 Manchester K. L. Randle P J & Young F G *J Endocr 19* 259 1959 (17, 21 22)
165 Marsh J B *J Clin Invest 31* 107 1952. (45)
166 Martin D B Renold, A. E. & Dagenais Y M *Lancet II* 76 1958 (13 25 26)
167 Martin D B Dagenais Y M Pfeiffer E. F & Renold, A. E. *Proc III Kongr*

Internat Diab Fed Dusseldorf, p 592, 1958 (26, 28)

168 Martin, D B, Dagenais, Y M & Renold, A E *in Anthoniades H N Hormones in human plasma* Churchill, London 1960 pag 78 (50)

169 Martinelli, M & Montanari, L *Giorn Gerontol 3* 584 1955 (23)

170 Metz, R *Diabetes 9* 89, 1960 (18, 20, 22, 63, 64, 65, 66, 77)

171 Mirsky, I A & Perisutti, G *Biochem Biophys Acta 50* 603, 1961 (30)

172 Moloney, P J *CIBA foundation colloquia on endocrinology* Vol 14 Churchill London 1962 pag 169 (33)

173 Montanari, L, Martinelli, M, Rossi, C A & Moruzzi, G *J A M A 147* 525, 1951 (23)

174 Morgan C R & Lazarow, A *Proc Soc Exp Biol Med 110* 29 1962 (32)

175 Morgan, C R & Lazarow, A *Diabetes 12* 115, 1963 (33, 34, 63, 64)

176 Oakley, W *III Kongr Internat Diab Federation* Dusseldorf 1958 pag 611 (45)

177 Ottaway J H *Brit med J II* 357, 1953 (23)

178 Ottaway, J H *J Endocr 21* 443, 1961 (23)

179 Ottaway, J H & Bulbrook R D *J Endocr 12* 50 1955 (23)

180 Oyama J & Grant, R L *Fed Proc 18* 297, 1959 (17)

181 Oyama J, Hagen H M & Grant R L *Proc Soc Exp Biol Med 104* 333, 1960 (17, 18, 20)

182 Park C R Brown, D H, Cornblath M Daughaday W H & Krahl M E *J Biol Chem 197* 151 1952 (23)

183 Peden, A S *Acta Endocr 18* 67 1955 (14)

184 Pellegrini, G Campagnari F Rodari, T & Rizzo S C *Boll Soc Med Chir (Pavia) 72* 1031 1958 (31 59)

185 Pellegrini G Maggi G Rodari T & Piotti *Boll Soc Med Chir (Pavia) 72* 1041 1958 (64)

186 Perlmutter M Weisenfeld S & Mufson M *Endocrinology 50* 442 1952 (16)

187 Pfeiffer E F, Renold A E Martin D B, Dagenais Y M Meakin J W Nelson, D H Shoemaker G & Thorn C W *III Kongr Internat Diab Federation,* Dusseldorf 1958 pag 298 (28, 65)

188 Pfeiffer, E F, Pfeiffer, M, Ditschuneit H & Chang Su Ahn *Ann N Y Acad Sci 82* 479, 1959 (25)

189 Pfeiffer, E F, Pfeiffer, M, Ditschuneit, H & Chang Su Ahn *Klin Wschr 37* 1239, 1959 (26, 68, 81)

190 Pfeiffer, E F, Ditschuneit, H & Ziegler, R *Klin Wschr 39* 415 1961 (28, 53 55, 81)

191 Phear, D N *Lancet II* 955, 1962 (52, 69, 81, 82)

192 Piazza, E G, Goodner, C L & Freinkel, N *Diabetes 8* 459 1959 (19)

193 Rabinowitz, D & Zierler, K L *J Clin Invest 41* 2173, 1962 (62, 82)

194 Rafaelsen, O J *Prize essay* Copenhagen University 1957 (17 19)

195 Rafaelsen, O J *Metabolism* 8 195 1959 (25)

196 Rafaelsen, O J *Rep IV Kongr Internat Diab Federation* Geneve pag 625, 1961 (14, 15, 52)

197 Rafaelsen O J *Acta physiol scand 61* 323 1964 (16)

198 Ramseir E B, Froesch E R Bally P & Labhardt A *Rep IV Kongr Internat Diab* Geneve 1961, pag 643 (26 28 53, 55 57)

199 Randle P J *Brit Med J I* 1237 1954 (15, 18 20 22 53 54)

200 Randle P J *Lancet I* 441 1954 (18 83)

201 Randle P J *The hypophyseal growth hormone nature and actions* Mc Graw Hill New York 1955 pag 143 (46)

202 Randle P J *J Endocr 14* 82 1956 (18, 20 21)

203 Randle P J *CIBA foundation colloquia on endocrinology* Vol 11 Churchill London 1957 pag 115 (14 50 51 52 53)

204 Randle P J *J Endocrin 15* 39 1957 (83)

205 Randle P J & Young F G *J Endocrin 13* 335 1956 (23 64 84)

206 Randle P J & Young F G *In Hormonal regulation of energy metabolism* Thomas Springfield 1957 pag 97 (23

207 Randle P J & Whitney J E *Nature* (London) *179* 1957 (22 23)

208 Randle P J & Howard Smith C *Biochem Biophys Acta 25* 442 1957 25

209 Randle P J & Taylor K W *Lancet II* 9 6 1958 (37)
210 Randle P J & Taylor K W *J Endocrin* 17 387 1958 (41)
211 Randle P J Garland P B Hales C N & Newsholme E A *Lancet I* 785 1963 (45)
212 Read C H & Bryan G T *Recent Progress Hormone Research 16* 187 1960 (23)
213 Renold A E Personal communication 1963 (28 33)
214 Renold A E & Martin D B *Ann N Y Acad Sc 71* 71 1957 (52)
215 Renold A E Zalnd G Jeanrenaud B & Boshell B R *Ann N Y Acad Sci 74* 490 1959 (31)
216 Renold A E Martin D B Dagenais Y M Steinke J Nickerson R J & Sheps M *J Clin Invest 39* 1487 1960 (26 28 30)
217 Riesser O *Biochem et Biophys Acta 1* 208 1947 (16 24)
218 Rizzo S C Rodar T Fratt L & Turba G *Fol Endocrinol 14* 1 1961 (69 81)
219 Rizzo S C Fra L & Turba, G *Fol Endocrinol* 14 163 1961 (72)
220 Rizzo S G Fattu L & Turba G *Comnale Biochem 11* 116 1962 (62)
221 Rizzo S G Fratt L & Turba G *Fol a Endocrinol 15* 83 1962 (52)
222 Roda T *Bell Soc Ital Biol Sper* m n ale 36 560 1960 (31 59)
223 Roda T Maccl G & Speccl a C *Ra Neurologa Veg t 15* 1 1960 (82)
224 Roda T Maggi G & Specch a G *Fola endocrinol 15* 6?5 1961 (72)
225 Reed B Felber J P Essold N & Vanot A *Sch Med Wschr 93* 1001 1963 36 53 56 6?)
226 Samaan N A Dempster W J Fraser R Please N W & Sullman D *J Endocrin 4* 63 1962 6 28 62 63 64 65
227 Samaan N A Sullman, D & Fraser R *Lancet II* 1 89 1962 (8 53 55 57 65
228 Samaan N A & Fraser R *Lancet II* 311 1963 (57 71
229 Samaan N A Fraser R & Dempster W J *Diabetes 1* 339 1963 (27 38 40 44 65
230 Samols E & Ryder J A *J Clin Invest 40* 2092 1961 (3? 56 62)
231 Samols E & Marks V *Brit Med J I* 507 1963 (56)
232 Sehon A H Kay M McGarry E & Rose B *J Lab Clin Med 45* 65 1955 (45)
233 Seltzer H S *Rep IV Kongr Internat Diab Ed Medicine & Hygiene* Geneve 1961 pag 950 (51 52 77)
234 Seltzer H S *J Clin Invest 41* 289 1962 (65)
235 Seltzer H S & Smith W L *Diabetes 8* 417 1959 (18 20 52 69 81)
236 Shaw W N & Shuey E W *Biochemistry* (Wash) 2 286 1963 (19 36 37 52 54 58 59)
237 Sheps M C Nickerson R J Dagenais Y M Steinke J Martin D B & Renold A E *J Clin Invest 39* 1499 1960 (26 52 55)
238 Snex, F M McMullen J & Hastings A B *J Biol Chem 198* 615 1952 (16)
239 Skom J H & Talmage D W *J Clin Invest 37* 83 1958 (32)
240 Slater J D H Samaan N A Fraser R & Sullman D *Brit Med J I* 1712 1961 (26 50)
241 Snedecor J G DeMeio R H & Pincus I J *Proc Soc Exp Biol Med 89* 336 1955 (24)
242 Soeldner S Personal communication 1963 (34)
243 Spellagy W N & Goetz F C *New Engl J Med 68* 988 1963 (56)
244 Stadie W C & Zapp J A jr *J Biol Chem 1 0* 55 1947 (16 17)
245 Stadie W C Haugaard N & Marsh J B *J Biol Chem 188* 173 1951 (?3)
246 Stadie W C Haugaard N Hills A G & Marsh J B *Am J Med Sc 218* 275 1949 (17 23)
247 Stadie W C Haugaard N & Marsh J B *Amer J Med Sc 218* 265 1949 (45)
248 Staub W *Schw Med Wschr 88* 830 1958 (18 20 26)
249 Steelman S L Oslapas, R & Busch R D *Proc Soc Exp Biol Med 105* 595 1960 (6 27)
250 Stein, W H & Moore S *J Biol Chem 211* 9 6 1954 (24)
251 Stein M Kipnis D M & Daughaday

Internat Diab Fed Düsseldorf, p 592, 1958 (26 28)
168 Martin, D B, Dagenais, Y M & Renold, A E *in Anthoniades H N Hormones in human plasma,* Churchill, London 1960 pag 78 (50)
169 Martinelli, M & Montanari, L *Giorn Gerontol 3* 584, 1955 (23)
170 Metz R *Diabetes 9* 89, 1960 (18, 20, 22, 63, 64, 65, 66, 77)
171 Mirsky, I A & Perisutti, G *Biochem Biophys Acta 50* 603, 1961 (30)
172 Moloney, P J *CIBA foundation colloquia on endocrinology* Vol 14 Churchill London 1962 pag 169 (33)
173 Montanari, L, Martinelli, M Rossi, C A & Moruzzi G *J A M A 147* 525, 1951 (23)
174 Morgan, C R & Lazarow, A *Proc Soc Exp Biol Med 110* 29, 1962 (32)
175 Morgan C R & Lazarow, A *Diabetes 12* 115, 1963 (33, 34, 63, 64)
176 Oakley, W *III Kongr Internat Diab Federation* Dusseldorf 1958, pag 611 (45)
177 Ottaway J H *Brit med J II* 357, 1953 (23)
178 Ottaway, J H *J Endocr 21* 443, 1961 (23)
179 Ottaway J H & Bulbrook R D *J Endocr 12* 50 1955 (23)
180 Oyama J & Grant, R L *Fed Proc 18* 297, 1959 (17)
181 Oyama J, Hagen, H M & Grant R L *Proc Soc Exp Biol Med 104* 333, 1960 (17, 18, 20)
182 Park C R, Brown D H Cornblath, M Daughaday, W H & Krahl, M E *J Biol Chem 197* 151 1952 (23)
183 Peden A S *Acta Endocr 18* 67, 1955 (14)
184 Pellegrini, G Campagnari F Rodari T & Rizzo S C *Boll Soc Med Chir (Pavia) 72* 1031 1958 (31 59)
185 Pellegrini G Maggi G Rodari T & Piotti *Boll Soc Med Chir (Pavia) 72* 1041, 1958 (64)
186 Perlmutter, M Weisenfeld S & Mufson M *Endocrinology 50* 442 1952 (16)
187 Pfeiffer, E F Renold A E Martin D B Dagenais Y M Meakin J W Nelson, D H Shoemaker G & Thorn G W *III Kongr Internat Diab Federation* Dusseldorf 1958 pag 298 (28, 65)
188 Pfeiffer, E F, Pfeiffer, M, Ditschuneit, H & Chang Su Ahn *Ann N Y Acad Sci 82* 479, 1959 (25)
189 Pfeiffer, E F, Pfeiffer, M, Ditschuneit H & Chang Su Ahn *Klin Wschr 37* 1239, 1959 (26 68, 81)
190 Pfeiffer, E F, Ditschuneit, H & Ziegler R *Klin Wschr 39* 415, 1961 (28 53, 55, 81)
191 Phear, D N *Lancet II* 955, 1962 (52 69, 81, 82)
192 Piazza, E G Goodner, C L & Freinkel, N *Diabetes 8* 459, 1959 (19)
193 Rabinowitz D & Zierler K L *J Clin Invest 41* 2173 1962 (62, 82)
194 Rafaelsen, O J *Prize essay,* Copenhagen University 1957 (17 19)
195 Rafaelsen, O J *Metabolism 8* 195, *1959 (25)*
196 Rafaelsen, O J *Rep IV Kongr Internat Diab Federation* Geneve pag 625 1961 (14, 15, 52)
197 Rafaelsen O J *Acta physiol scand 61 323 1964 (16)*
198 Ramseir E B, Froesch E R Bally P & Labhardt A *Rep IV Kongr Internat Diab* Geneve 1961 pag 643 (26 28 53, 55, 57)
199 Randle, P J *Brit Med J I* 1237 1954 (15 18 20 22, 53 54)
200 Randle P J *Lancet I* 441 1954 (18 83)
201 Randle, P J *The hypophyseal growth hormone nature and actions* Mc Graw Hill New York 1955 pag 143 (46)
202 Randle P J *J Endocr 14* 82 1956 (18 20 21)
203 Randle, P J *CIBA foundation colloquia on endocrinology* Vol 11 Churchill London 1957 pag 115 (14 50 51 52 53
204 Randle P J *J Endocrin 15* 39 1957 (83)
205 Randle P J & Young F G *J Endocrin 13* 335 1956 (23 64 84)
206 Randle P J & Young F G *In Hormonal regulation of energy metabolism* Thomas Springfield 1957 pag 97 23
207 Randle P J & Whitney J E *Nature* (London) *179* 1957 (22 23)
208 Randle P J & Howard Smith C *Biochem Biophys Acta 25* 142 1957 25

209 Randle P J & Taylor K W *Lancet II* 996 1958 (37)
210 Randle P J & Taylor K W *J Endocrin 17* 387 1958 (41)
211 Randle P J Garland P B Hales C N & Newsholme E A *Lancet I* 785 1963 (45)
212 Read C H & Bryan G T *Recent Progress Hormone Research 16* 187 1960 (23)
213 Renold A E *Personal communication* 1963 (28 33)
214 Renold A E & Martin D B *Ann N Y Acad Sci 71* 71 1957 (52)
215 Renold A E Zahnd G Jeanrenaud B & Boshell B R *Ann N Y Acad Sci 74* 490 1959 (31)
216 Renold A E Martin D B Dagenais Y M Steinke J Nickerson R J & Sheps M *J Clin Invest 39* 1487 1960 (26 28 30)
217 Riesser O *Biochem et Biophys Acta I* 208 1947 (16 24)
218 Rizzo S C Rodari T Fratti L & Turba C *Fol Endocrinol 14* 1 1961 (69 81)
219 Rizzo S C Fratti L & Turba C *Fol Endocrinol 14* 169 1961 (72)
220 Rizzo S G Fratti L & Turba G *Giornale Biochem 11* 116 1962 (62)
221 Rizzo S G Fratti L & Turba G *Folia Endocrinol 15* 83 1962 (52)
222 Rodari T *Boll Soc Ital Biol Sperimentale 36* 560 1960 (31 59)
223 Rodari T Macchi G & Specchia G *Rass Neurologia Veget 15* 1 1960 (8?)
224 Rodari T Maggi C & Specchia G *Folia endocrinol 15* 625 1961 (72)
225 Ruedi B Felber J P Essold N & Vanotti A *Schw Med Wschr 93* 1001 1963 (36 53 56 62)
226 Samaan N A Dempster W J Fraser R Please N W & Stillman D *J Endocrin 24* 263 1962 26 38 62 63 64 65
227 Samaan N A Stillman D & Fraser R *Lancet II* 1288 1962 28 53 55 57 62 65
228 Samaan N A & Fraser R *Lancet II* 311 1963 (57 71)
229 Samaan N A Fraser R & Dempster W J *Diabetes 12* 339 1963 (27 38 40 44 65
230 Samols E & Ryder J A *J Clin Invest 40* 2092 1961 (32 56 62)
231 Samols, E & Marks V *Brit Med J I* 507 1963 (56)
232 Sehon A H Kay M McGarry E & Rose B *J Lab Clin Med 45* 765 1955 (45)
233 Seltzer H S *Rep IV Kongr Internat Diab Ed Medicine & Hygiene* Geneve 1961 pag 950 (51 57 77)
234 Seltzer H S *J Clin Invest 41* 289 1962 (65)
235 Seltzer H S & Smith W L *Diabetes 8* 417 1959 (18 20 52 69 81)
236 Shaw W N & Shuey E W *Biochemistry* (Wash) 2 286 1963 (19 36 37 52 54 58 59)
237 Sheps M C, Nickerson R J Dagenais Y M Steinke J Martin D B & Renold, A E *J Clin Invest 39* 1499 1960 (26 52 55)
238 Sinex, F M McMullen J & Hastings A B *J Biol Chem 198* 615 1952 (16)
239 Skom J H & Talmage D W *J Clin Invest 37* 783 1958 (32)
240 Slater J D H Samaan N A Fraser R & Stillman D *Brit Med J I* 1712 1961 (26 50)
241 Snedecor J G DeMeio R H & Pincus A J *Proc Soc Exp Biol Med 89* 396 1955 (24)
242 Soeldner S *Personal communication* 1963 (34)
243 Spellagy W N & Goetz F C *New Engl J Med 268* 968 1963 (56)
244 Stadie W C & Zapp J A jr *J Biol Chem 170* 55 1947 (16 17)
245 Stadie W C Haugaard N & Marsh J B *J Biol Chem 188* 173 1951 (23)
246 Stadie W C Haugaard N Hills A G & Marsh J B *Am J Med Sc 218* 275 1949 (17 23)
247 Stadie W C Haugaard N & Marsh J B *Amer J Med Sc 218* 265 1949 (45)
248 Staub M *Schw Med Wschr 88* 830 1958 (18 20 26)
249 Steelman S L Oslapas R & Busch R D *Proc Soc Exp Biol Med 103* 595 1960 (26 27)
250 Stein, W H & Moore S *J Biol Chem 211* 926 1954 (4)
251 Stein M Kapnis D M & Daughaday

W H *J Lab Clin Med 60* 1022, 1962 (84)
252 Steinke, J *Personal communication* 1960 (50)
253 Steinke, J, Taylor, K W & Renold, A E *Lancet I* 30, 1961 (68, 81)
254 Steinke, J, Taylor, K W, Gundersen, K & Renold, A E *Rep IV Kongr Internat Diab Fed* Geneve pag 632, 1961 (50, 52, 53, 55, 68, 69, 81)
255 Steinke, J, Camerini, R, Marble, A & Renold, A E *Metabolism 10* 707, 1961 68)
256 Steinke, J, Renold, A E, Knaack, J, & Anthoniades, H N *J clin Invest 40* 1083, 1961 (77)
257 Steinke, J, Sirek, A Lauris, V, Lukens, F D W & Renold, A E *J clin Invest 41* 1699 1962 (27, 63, 64)
258 Stewart, G A *Brit Med Bull 16* 197, 1960 (15)
259 Takeuchi, S, Ohashi, S & Kobayashi, Y *J Pharm Exp Ther 119* 439, 1957 (18, 19, 20, 21, 64)
260 Takeuchi, S, Tagagi, M & Kobayashi, Y *Endocr Jap 8* 57, 1961 (64)
261 Taylor, K W *Rep IV Kongr Internat Diab Fed* Geneve pag 640, 1961 (44)
262 Taylor, K W *Brit Med J I* 511, 1963 (72, 73, 81)
263 Taylor, K W & Randle, P S *J Endocrin 19* 221, 1959 (37)
264 Taylor, K W Vargas, L & Randle, P J *Lancet I* 1313 1960 (44)
265 Tuerkischer E & Wertheimer, E *J Biol Chem 42* 603, 1948 (16, 24 45)
266 Unger, R H, Eisentraut, A M McCall M S & Madison, L L *J Clin Invest 41* 682, 1962 (24)
267 Utiger, R D, Parker, M L & Daughaday W H *J Clin Invest 41* 254, 1962 (23)
268 Valk, A T & Price, H L *J Clin Invest 35* 837, 1956 (24)
269 Vallance Owen, J Rep *III Kongr Internat Diab Federation* Dusseldorf 1958, pag 587 (21)
270 Vallance Owen, J Rep *III Kongr Internat Diab Federation* Dusseldorf 1958, pag 605 (42)
277 Vallance Owen, J & Hurlock B *Lancet I* 68 1954 (18 22 52)
272 Vallance Owen, J Hurlock B & Please, N W *Lancet I* 983 1954 (20)
273 Vallance Owen, J, Hurlock, P & Please, N W *Lancet II* 583, 1955 (42, 78, 81)
274 Vallance Owen, J & Feinberg in Conference on insulin activity in blood and tissue fluids' Nat Inst Health Bethesda Md 1957 (22)
275 Vallance Owen, J & Lukens, F D W *Endocrinology 60* 625, 1957 (22, 28, 46, 64)
276 Vallance Owen, J, Dennes, E & Campbell, P N *Lancet II* 336, 1958 (43)
277 Vallance Owen, J, Dennes, E & Campbell, P N *Lancet II* 596, 1958 (42, 43)
278 Vallance Owen, J, Joplin, G F & Fraser, R *Lancet II* 584, 1959 (81)
279 Vallance Owen, J & Wright P H *Phys Rev 40* 219, 1960 (11, 19)
280 Vallance Owen, J & Lilley, M D *Lancet I* 804, 1961 (43)
281 Vallance Owen, J & Lilley, M D *Lancet I* 806 1961 (43)
282 Vallance Owen, J & Ashton, W L *Lancet I* 1226, 1963 (43)
283 Vargas, L, Taylor, K W & Randle, P J *Biochem J 77* 43, 1960 (35)
284 Villee, C A & Hastings, A B *J Biol Chem 179* 673, 1949 (16)
285 Villee, C A, White, V K & Hastings, A B *J Biol Chem 195* 287, 1952 (16)
286 Walås E & Walås, C *Biochem Biophys Acta 20* 77 1956 (24)
287 Wardlaw, A C & Moloney, P J *Can J Biochem Physiol 39* 695, 1961 (20)
288 Weil Malherbe, H & Bone, A D *Biochem J 58* 132, 1954 (24)
289 Weil Malherbe H & Bone A D *Biochem J 67* 65 1957 (24)
290 Weinges, K F & Wörner H *Klin Wschr 39* 238 1961 (26 27, 53)
291 Wiberg G S & Jessup D C *Diabetes 10* 198, 1961 (17, 18 20 21)
292 Willebrands A F *Clin Chim Acta 5* 508, 1960 (44 76 81)
293 Willebrands A F & Groen J *Adv Intern Med 6* 331 1954 (11)
294 Willebrands A F & Groen J *Diabetes 5* 378 1956 (18, 20 53 81)
295 Willebrands A F v d Geld H & Groen J *Diabetes 7* 119 1958 (18 19 20 50 53)

296 Williams R H Tyberghein P M & Nielsen, L. *Metabolism 6* 311, 1957 (25)
297 Winegrad A. I & Renold, A E *J Biol Chem 233* 267, 1958 (25)
298 Winegrad A I & Renold A. E *J Biol Chem 233* 273 1958 (25)
299 Winegrad A I Shaw W N Lukens F D W & Stadie W C *J Biol Chem 234* 3111 1959 (29 30)
300 Withney J E & Young F G *Biochem J 66* 645 1957 (46)
301 Wrenshall G A, Bogoch A. & Ritchie R C *Diabetes I* 87, 1952 (77)
302 Wright P H *Lancet II* 621 1957 (18 20 52 81)
303 Wright P H *Biochem J 71* 633 1959 (22)
304 Wright, P H *Lancet I* 951 1960 (50 51 52 53 83)
305 Worner H & Weinges K F *Klin. Wschr 39* 243 1961 (25 26 29)
306 Zahnd G Steinke J & Renold A. E *Helv Med Acta 27* 703, 1960 (84)
307 Yalow R S & Berson, S A. *Nature 184* 1648 1959 (31)
308 Yalow R. S & Berson S A *J Clin Invest 39* 1157, 1960 (34 50 56 70 83)
309 Yalow, R S & Berson S A. *Amer J Med 29* 1 1960 (11)
310 Yalow R. S & Berson S A. *Amer J Med 31* 882 1961 (34 45)
311 Yankelowitz T Massry S & Gitter S *Diabetes 5* 457 1956 (45)
312 Young F G *Endocrinology 73* 654 1963 (36)
313 Ørskov H C *Personal communication* 1964 (33 56)

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ACTA MEDICA
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SUPPLEMENTUM 442

CLINICAL STUDIES ON KIDNEY FUNCTION WITH RADIOACTIVE SODIUM DIATRIZOATE (HYPAQUE®)

BY

TORSTEN DENNEBERG

Accompanies Vol. 179

LUND 1965

ACTA MEDICA SCANDINAVICA

has been published since 1919 as a continuation of Nordiskt Medicinskt Arkiv, founded in 1869 by Axel Key The first volume of Acta Medica Scandinavica is therefore numbered LII (52)

The chief editors have been Axel Key 1869—1900, C G Santesson 1901—1915, I Holmgren 1916—1957 and Birger Strandell 1958 to date

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ACTA MEDICA SCANDINAVICA
SUPPLEMENTUM 442

FROM THE DEPARTMENT OF INTERNAL DISEASES, MALMÖ GENERAL HOSPITAL, MALMÖ
UNIVERSITY OF LUND
HEAD: PROFESSOR JAN WALDENSTRÖM

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Translated by L. James Brown

Printed in Sweden

LUND 1965
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CONTENTS

CHAPTER I

INTRODUCTION

Hypaque© (sodium 3 5 diacetamido 2 4 6 triiodobenzoate or sodium diatrizoate) has been widely used in the last decade in excretion urography Labelled with ^{131}I the substance (^{131}I Hypaque) has also been used in clearance studies and external renal measurements (*radioisotope renogram*) Despite this extensive use the relative roles of glomerular and tubular function in the excretion of ^{131}I Hypaque are still obscure Better knowledge of the disappearance of the substance from the plasma, its renal and extrarenal excretion as well as its clearance would be useful in the evaluation of the possibility of using ^{131}I Hypaque as a substitute for inulin in the measurement of glomerular filtration and as a suitable substance for single injection clearance tests and in the interpretation of renograms

rather low incidence of untoward reactions and the very good visualization it offers (Lowman et al. 1955 Moore & Mayer 1955 Harrow 1956 and others) So far, only few cases have been reported in which side effects could be ascribed to the Hypaque (Berlyne & Berlyne 1962 Hansson & Lindholm 1963)

While the excretory mechanism of the previously mentioned contrast media such as Diodrast and Hippuran is well known *opinions differ about* the mode of excretion of triodinated benzoic compounds The first reports on the renal excretion of Hypaque and Urografin were based on animal experiments Langecker et al (1954) who used dogs showed that at plasma concentrations about or below 1 mg/100 ml the Urografin/inulin clearance quotient is more than 1 0, indicating that Urografin is also excreted by the tubules They concluded that in a dose of 10 g (i e amounts used for excretion urography) Urografin is *probably excreted mainly by glomerular filtration* but when the plasma level is low also by the tubules

Knoefel & Huang (1956) and Knoefel et al (1961) studied several iodinated derivatives and found that substances with an acylamido group in the 3 position or in 3 and 5 positions were excreted by the tubules Hypaque was one of these substances and was *found to* have a certain maximal rate of tubular excretion

McChesney & Hoppe (1957) who used doses of Hypaque in amounts employed in roentgen examinations, found that its clearance was independent of plasma concentrations between *90—1200 mg/100 ml and* that the clearance with doses of this size closely resembled that of inulin They also noted that Hypaque was apparently not influenced by the simultaneous transport of PAH through the tubules These findings were interpreted as indicating that in the doses used Hypaque was excreted mainly by glomerular filtration These animal experiments with stable Hypaque seemed to show *that in small doses it is eliminated by* tubular excretion as well as by glomerular filtration, but in the large doses used for roentgenography it is excreted mainly by glomerular filtration

The introduction of radioactive isotope techniques improved the possibilities of studying renal function (Oeser & Billion 1952 Wossidlo 1952) Oeser & Billion (1952) used ^{131}I labelled Uroselectan in their investigations of renal function and measured its excretion in the urine by determining the beta ray activity after a single intravenous injection *Billion & Schlungbaum (1955)* elaborated the technique and found that ^{131}I labelled Perabrodil® (iodopyracet) could be used also in clearance studies for estimating renal function and renal plasma flow with the constant intravenous infusion technique The gamma radiation was utilized for external measurements over the kidneys by Kimbel (1956) who compared ^{131}I Urografin and ^{131}I Perabrodil The method of external measurement over the kidneys was introduced and systematically developed as a clinical procedure *(radioisotope renogram)* by Taplin et al (1956)

SURVEY OF LITERATURE

A *Iodinated contrast media used in kidney studies*

Several iodinated organic contrast media have proved useful in the investigation of renal function and renal blood flow The first iodinated organic substance used for excretion urography was that described by Swick (1929), viz Uroselectan® (sodium iodomethamate) Swick (1933) also introduced Hippuran® (sodium o iodohippurate) as a contrast medium for excretion urography Several other contrast media were studied and tried but iodopyracet, Diodrast® (3,5 diiodo-4 pyridone N acetic acid diethanolamine) soon proved the most suitable and was the medium most frequently used during the following 20 years, it was also widely used in renal clearance studies (Elsom et al 1936, Smith et al 1938 and others) Para amino hippuric acid (PAH), whose clearance was identical with that of Diodrast and Hippuran, could be determined chemically in a simple way, which was one of the chief reasons why this substance was most widely used (Smith et al 1945)

In the beginning of the 1950s several iodinated acylaminobenzoic compounds with a still higher iodine content were studied by Wallingford et al (1952), who found sodium acetrizoate UroKon® (sodium 3 acetamido 2 4,6 triiodobenzoate) to be a suitable urographic agent A chemically closely related substance is sodium diatrizoate, Hypaque (sodium 3,5 diacetamido 2,4 6 triiodobenzoate) which was synthetized by Larsen et al (1954) in U S A This latter compound was described independently at the same time in Germany by Langecker et al (1954), who carried out a clinical trial with a mixture of the sodium- and N methyl glucamine salts (Urografin® in Europe, Renografin® in U S A)

The high degree of radiopacity of these substances, their low systemic and local tissue toxicity, prompt complete elimination by the kidneys and insignificant pharmaco dynamic side effects led to further improvement of the roentgenological diagnosis of renal diseases (Langecker et al 1954, Hoppe et al 1956 Knoefel & Huang 1956 Hoppe 1959 Lindgren & Törnell 1958 Lindgren 1961 and others)

In most clinical studies in which comparisons have been made of various renal contrast media such as Hypaque UroKon and Diodrast Hypaque has proved superior because of the

This rapid elimination of the substance from the blood agreed well with the corresponding rapid elimination by the kidneys. No substantial difference in excretion rate appeared to occur when non radioactive Hypaque or Urografin was used in a dose of 0.5 g/kg or tracer doses. The rapid excretion by the kidneys may be characterized by the following values in dogs: 67 per cent of the dose was excreted in 2 hours, 88 per cent in 8 hours and 92 per cent in 24 hours (McChesney & Hoppe 1957) which was in good agreement with the 24 hour value of 95 per cent reported by Blaufox et al (1963 d).

Kimbel & Borner (1955) found that after injection of labelled Urografin into rats the concentration in all parenchymatous organs and in the blood fell rapidly with the exception of the small and large intestines where about 10 per cent of the dose was still present after 3 hours. After 24 hours it was no longer possible to demonstrate any activity in the animals except in the intestines. McChesney & Hoppe (1957) made similar findings in investigations with non radioactive Hypaque in cats. The pattern of the tissue distribution suggested that most of the Hypaque was located in the extracellular fluid and with time Hypaque was rather rapidly removed and transported to the kidneys for excretion. No special predilection for any other organ or tissue was observed.

Langecker et al (1954) found 0.5 and 0.7 per cent of the dose of non radioactive Urografin in the bile in rabbits after 2 and 6 hours, respectively. These findings agreed with those reported by Hansson & Lindholm (1963) in rabbits and Blaufox et al (1963 d) in dogs while zum Winkel & DeMaria (1961) and Kimbel (1963) reported somewhat higher values with addition of carrier to the radioactive substance in rats. While the excretion in the bile was insignificant in normal rabbits it was as high as 25 per cent in nephrectomized animals. The values with low biliary excretion agreed well with those reported by McChesney & Hoppe (1957) who found that the faecal excretion in dogs was 0.7 per cent in 24 hours and 2 per cent in 72 hours.

Investigations on animals have thus shown that under normal conditions Hypaque or Urografin is eliminated rapidly from the blood by the kidneys and that no substantial uptake occurs in any other organ and that there is no significant extrarenal excretion. The picture is quite different in experimental anuria, when the excretion in the bile and by the intestines increases considerably.

Studies in human beings

Since the blood concentration curves and the disappearance rate of ^{131}I Hypaque as well as its distribution and its renal excretion will be discussed in detail in Chapter VI, suffice it here to say that the various investigators have analysed the blood curves with the help of slope analysis (Schlungbaum & Billion 1956 b, Denneberg et al 1961 a, Stokes & Ter Pogossian 1964)

These ^{131}I labelled contrast media have been used 1) as substitute for a corresponding non radioactive medium in clearance studies, 2) for external measurement over the kidneys (radio isotope renogram), 3) for renal scanning and scintigraphy and 4) in combinations of the above mentioned examinations for determination of total renal function and of the performance of each kidney separately

Experimental investigations on animals and trials on human beings with the single injection technique for elucidating the distribution and kinetics of the radioactive substances have been reported by several authors In these experiments the blood concentration curves and corresponding external curves over different parts of the body (head, foot, heart and kidneys) have been analysed Animal experiments with direct measurements of radioactivity of organs or with autoradiographic technique have also been performed (Kimbel & Börner 1955, Denneberg et al 1960, Magnusson 1960 1962, zum Winkel & DeMaria 1961, Schlungbaum 1962 zum Winkel 1964) The single injection technique has been used to study the mechanism of the renal excretion of the substance (Bianchi 1961 Denneberg et al 1961 a, Bianchi & Toni 1962 Gott et al 1962 Blaufox et al 1963 b d Meschan et al 1963 a, c)

With the aid of the continuous intravenous infusion technique according to the principles described by Homer Smith (1951) labelled and unlabelled substances have been compared regarding their renal excretion The purpose was to substitute the non radioactive substance by the radioactive agent for determination of glomerular filtration, tubular secretion and renal blood flow (Billion & Schlungbaum 1955, Kinter & Pappenheimer 1956, Schlungbaum & Billion 1956 a, Bergstrom et al 1959, Block & Burrows 1960, Parker & Beierwalles 1960, Bianchi & Zampieri 1961, Burbank et al 1961, Schwartz & Madeloff 1962)

The double isotope technique using labelling of substances with either ^{125}I or ^{131}I opened further possibilities of using these substances in the diagnosis of renal diseases (Stokes & Ter Pogossian 1964) For this purpose several investigations were made with labelled Diodrast and Hippuran for determining renal plasma flow, and various labelled triiodinated substances, particularly Hypaque (Renografin or Urografin), were studied for their value as substitutes for inulin in the measurement of glomerular filtration

B *Distribution and excretion of sodium diatrizoate (Hypaque, Renografin, Urografin)*

Experiments in animals

Kimbel & Borner (1955) found that the blood curves obtained after a single injection of radioactive Urografin fell rapidly during the first 3 hours and after 24 hours it was hardly possible to demonstrate any activity in the blood Similar findings have been reported by Hansson & Lindholm (1963) with radioactive Hypaque in rabbits

This rapid elimination of the substance from the blood agreed well with the corresponding rapid elimination by the kidneys. No substantial difference in excretion rate appeared to occur when non radioactive Hypaque or Urografin was used in a dose of 0.5 g/kg or tracer doses. The rapid excretion by the kidneys may be characterized by the following values in dogs: 67 per cent of the dose was excreted in 2 hours, 88 per cent in 8 hours and 92 per cent in 24 hours (McChesney & Hoppe 1957) which was in good agreement with the 24 hour value of 95 per cent reported by Blaufox et al (1963 d).

Kimbel & Borner (1955) found that after injection of labelled Urografin into rats the concentration in all parenchymatous organs and in the blood fell rapidly with the exception of the small and large intestines where about 10 per cent of the dose was still present after 4 hours. After 24 hours it was no longer possible to demonstrate any activity in the animals except in the intestines. McChesney & Hoppe (1957) made similar findings in investigations with non radioactive Hypaque in cats. The pattern of the tissue distribution suggested that most of the Hypaque was located in the extracellular fluid and with time Hypaque was rather rapidly removed and transported to the kidneys for excretion. No special predilection for any other organ or tissue was observed.

Langecker et al (1954) found 0.5 and 0.7 per cent of the dose of non radioactive Urografin in the bile in rabbits after 2 and 6 hours respectively. These findings agreed with those reported by Hansson & Lindholm (1963) in rabbits and Blaufox et al (1963 d) in dogs while zum Winkel & DeMaria (1961) and Kimbel (1963) reported somewhat higher values with addition of carrier to the radioactive substance in rats. While the excretion in the bile was insignificant in normal rabbits it was as high as 25 per cent in nephrectomized animals. The values with low biliary excretion agreed well with those reported by McChesney & Hoppe (1957) who found that the faecal excretion in dogs was 0.7 per cent in 24 hours and 2 per cent in 72 hours.

Investigations on animals have thus shown that under normal conditions Hypaque or Urografin is eliminated rapidly from the blood by the kidneys and that no substantial uptake occurs in any other organ and that there is no significant extrarenal excretion. The picture is quite different in experimental anuria when the excretion in the bile and by the intestines increases considerably.

Studies in human beings

Since the blood concentration curves and the disappearance rate of ^{131}I Hypaque as well as its distribution and its renal excretion will be discussed in detail in Chapter VI suffice it here to say that the various investigators have analysed the blood curves with the help of slope analysis (Schlungbaum & Billion 1956 b, Denneberg et al 1961 a, Stokes & Ter Pogossian 1964)

These ^{131}I-labelled contrast media have been used 1) as substitute for a corresponding non radioactive medium in clearance studies, 2) for external measurement over the kidneys (radioisotope renogram), 3) for renal scanning and scintigraphy and 4) in combinations of the above mentioned examinations for determination of total renal function and of the performance of each kidney separately

Experimental investigations on animals and trials on human beings with the single injection technique for elucidating the distribution and kinetics of the radioactive substances have been reported by several authors In these experiments the blood concentration curves and corresponding external curves over different parts of the body (head, foot, heart and kidneys) have been analysed Animal experiments with direct measurements of radioactivity of organs or with autoradiographic technique have also been performed (Kimbel & Börner 1955, Denneberg et al 1960 Magnusson 1960, 1962 zum Winkel & DeMaria 1961, Schlungbaum 1962, zum Winkel 1964) The single injection technique has been used to study the mechanism of the renal excretion of the substance (Bianchi 1961, Denneberg et al 1961 a Bianchi & Toni 1962 Gott et al 1962 Blaufox et al 1963 b d, Meschan et al 1963 a c)

With the aid of the continuous intravenous infusion technique according to the principles described by Homer Smith (1951) labelled and unlabelled substances have been compared regarding their renal excretion The purpose was to substitute the non radioactive substance by the radioactive agent for determination of glomerular filtration, tubular secretion and renal blood flow (Billion & Schlungbaum 1955, Kinter & Pappenheimer 1956, Schlungbaum & Billion 1956 a, Bergström et al 1959, Block & Burrows 1960 Parker & Beierwaltes 1960 Bianchi & Zampieri 1961, Burbank et al 1961, Schwartz & Madeloff 1962)

The double isotope technique using labelling of substances with either ^{1} I or ^{131}I opened further possibilities of using these substances in the diagnosis of renal diseases (Stokes & Ter Pogossian 1964) For this purpose several investigations were made with labelled Diodrast and Hippuran for determining renal plasma flow and various labelled triiodinated substances particularly Hypaque (Renografin or Urografin), were studied for their value as substitutes for inulin in the measurement of glomerular filtration

B *Distribution and excretion of sodium diatrizoate (Hypaque, Renografin, Urografin)*

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The double isotope technique using labelling of substances with either ^{125}I or ^{131}I opened further possibilities of using these substances in the diagnosis of renal diseases (Stokes & Ter Pogossian 1964) For this purpose several investigations were made with labelled Diodrast and Hippuran for determining renal plasma flow, and various labelled triiodinated substances particularly Hypaque (Renografin or Urografin), were studied for their value as substitutes for inulin in the measurement of glomerular filtration

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the filtered ^{131}I Hypaque was re ab sorbed during high urine flow rate The increase in ^{131}I Hypaque clearance during osmotic diuresis was believed to reflect re absorption of ^{131}I Hypaque in the proximal tubules They concluded, however, that ^{131}I Hypaque clearance in dogs is predominantly a function of glomerular filtration

While the above mentioned authors compared ^{131}I Hypaque with creatinine Meschan et al (1963 a) compared radioactive Renografin without addition of carrier and inulin They found that the clearance quotient was between 0 92 and 1 15 in 13 separate tests on 5 dogs The mean of the clearance quotients was 1 01 They therefore concluded that ^{131}I Renografin is a good substitute for inulin in measurement of glomerular filtration Investigations on dogs have thus given contradictory results but the possibility of a partial tubular secretion and re absorption can not be excluded Since *no experiments have been performed with inhibitors of tubular activity* the problem is not definitely solved

Studies in human beings

The mechanism of renal excretion of Hypaque in human beings has been studied by a number of investigators using different isotope methods (Bianchi 1961 Bianchi & Zampiere 1961 Dennebergh et al 1961 a Schlungbaum 1962 Bianchi & Toni 1963 Tauxe et al 1964 Morris et al 1965) They all found ^{131}I Hypaque to have a lower renal clearance value than substances of the type ^{131}I Diodrast PAH and ^{131}I Hippuran but opinions differ as to whether it is excreted by glomerular filtration alone or also partially by tubular secretion These problems will be discussed in detail in Chapter VII

D *Studies on interaction between sodium diatrizoate and serum proteins*

In the investigation of renal function knowledge of the protein binding of the test substance used is of importance Homer Smith (1951) pointed out that although protein binding may retard diffusion from the peritubular capillaries into the interstitial fluid it will not render the substance unavailable for tubular excretion because the protein complex may be rapidly reversible Reduction of concentration of the substance in the interstitial fluid on uptake by the tubules is immediately compensated by diffusion of the substance from the capillaries with consequent reduction of its concentration in the plasma In principle all of the substances contained within the plasma may dissociate and escape by diffusion before the blood emerges from the peritubular capillaries and this does in fact occur Proof of this is shown by identical clearance rates of Diodrast Hippuran and PAH though each of these substances exhibits a different degree of protein binding When calculating the amount of a substance filtered through the glomeruli on the other hand the binding on protein is believed to be of importance

and all the investigators found ¹³¹I-Hypaque or ¹³¹I-Urografin to be rapidly eliminated from the blood Examinations of the excretion rate of ¹³¹I-Urografin gave values compatible with the rapid elimination from the blood (Langecker et al 1954, Kimbel & Borner 1955, Schlungbaum & Billion 1956 b) The excretion by extra-renal routes were negligible or insignificant (bile, gastric juice, saliva), the faecal excretion was between 1—2 per cent (Kimbel & Börner 1955, Schlungbaum & Billion 1956 b)

C *Renal excretion mechanism of sodium diatrizoate (Hypaque, Renografin, Urografin)*

Experiments in animals

The mechanism of the renal excretion of Hypaque has been studied in animals with various isotope methods The majority of investigators have concluded that Hypaque is excreted mainly by glomerular filtration but as mentioned above findings by Langecker et al (1954) argued for an excretion by the tubules when non radioactive Urografin was given in doses about or below 1 mg/100 ml In comparison between radioactive Diodrast and Urografin Willenbrink & Kimbel (1959) found that both were excreted by aglomerular marine teleost fishes, but that the excretion of Diodrast was much greater (during 24 hours 16 2 per cent and 3 1 per cent respectively) In rabbits however they found that ¹³¹I Urografin clearance was unchanged at plasma concentrations of 1—1000 mg/100 ml and that saturation of the tubules with PAH did not influence the rate of excretion of ¹³¹I Urografin They therefore thought that in this range of plasma concentrations ¹³¹I Urografin was excreted only by glomerular filtration

In cats Denneberg et al (1960) studied the tissue distribution and renal excretion of radioactive Hypaque, Diodrast and Urokon with autoradiography and external measurements They found that the three substances accumulated in the renal cortex and in the medullary zone and were rapidly eliminated into the pelvis All the substances accumulated in the tubules, but they differed in the rate of excretion to the renal pelvis They concluded that ¹³¹I Hypaque was probably excreted by the same mechanism as ¹³¹I Diodrast in the cat

Woodruff & Malvin (1960), who used stop flow analysis and the infusion clearance technique with ¹³¹I Hypaque in dogs, found the clearance to be independent of the plasma level Judging from the clearance data a small amount of the filtered mass appeared to be reabsorbed However stop flow and protein binding studies revealed ¹³¹I Hypaque to be handled exactly like creatinine by the kidney Stokes et al (1962) found no significant change in the quotient ¹³¹I Hypaque/creatinine with plasma concentrations between 0 02 and 94 mg/100 ml The quotient varied between 0 52 and 0 89 at the above plasma levels but increased up to 0 95 during osmotic diuresis Approximately 20 per cent of

reported their first experiences with the method in animal experiments and in human beings with various renal diseases Winter (1956 1957) was the first to compare the *isotope* renogram with roentgenographic findings and the results of conventional renal function tests in a clinical series He described the test as a screening test for demonstrating unilateral renal injury in patients with hypertension

The now voluminous literature on radioisotope renograms in the investigation of various renal diseases has been reviewed by Magnusson (1962) Winter (1963) and zum Winkel (1964) These publications survey the experimental and clinical development of the technique One of the most important factors in the development of the renogram test was the choice of a suitable test substance *Various label*led iodinated substances have been tried such as Diodrast PAH Hippuran as well as substances of the type triiodobenzoic (*Hypaque Urografin Renografin* Miokon and Urokon)

Taplin et al (1956) found ^{131}I Urokon to be useful in animal experiments but not to be suitable for clinical use According to these authors ^{131}I Diodrast with its high renal clearance appeared to be the most suitable sub*stance But this substance like di iodo* PAH ^{131}I is excreted not only by the kidneys but also partly by the liver and the biliary ducts. The extent of this excretion is such that the radioactivity in the liver affects the external renal measurements on the right side and thus *interferes* with the comparison between the two sides (Winter & Taplin 1958 *Denneberg & Hedenskog* 1959 a, Dollery 1960 Bianchi 1961)

In trials with ^{131}I Hypaque the excretion by the liver and biliary ducts *did not prove disturbing but the ascent* of the second segment and the fall of the third segment were more gentle than in curves obtained with ^{131}I Diodrast (Winter & Taplin 1958) ^{131}I-Hippuran which was introduced by Nordyke et al (1960) proved to be comparable to ^{131}I Diodrast regarding its uptake by the kidneys but without the undesired excretion by the hepatic *and biliary ducts This substance has* therefore been used most widely in recent years for renography

While the choice of the test substance may be regarded as decided *some factors in the technique describ*ed by Taplin et al (1956) have been modified by other investigators in order to improve the accuracy of the *method and to facilitate the measuring procedure Factors of importance* that have received attention in these modifications are the position of the patient during the measurements (sitting prone or supine) the localisation of the kidneys for exact adjustment *of the* detectors and collimation of the crystal and detector In addition to these technical variables simultaneous external measurements over the urinary bladder (renocystogram) or selected parts of the body (heart, head foot) have *contributed* to our knowledge of the elimination of the substance from the blood and its arrival in the blad

According to Homer Smith (1951), the most suitable methods for studying plasma protein binding of renal test agents is ultrafiltration and dialysis through membranes impermeable to plasma proteins It is, however, remarkable that investigators have obtained widely discrepant results (Smith & Smith 1938, Block & Burrows 1960, Magnusson 1962 and others) This also holds for Hypaque and Urografin Lasser et al (1962) underlined the large difference in this respect between species, which may explain the discrepancies between the results obtained by various investigators, but it also appeared probable that the cause of the differences should be sought in differences between the methods used Thus Langecker et al (1954), who used ^{131}I Urografin *in vitro*, found with the technique of ultrafiltration at plasma levels from 6 5 to 42 mg per cent values varying from 24 8 to 31 8 and 26 3 to 36 0, respectively, in the percentage bound to the plasma proteins in dogs Also Knoefel & Huang (1956) used ultrafiltration in experiments in dogs and found that 11—24 per cent of Hypaque was bound to serum proteins On ultrafiltration with semipermeable membranes or equilibrium dialysis other investigators such as Woodruff & Malvin (1961) Lasser et al (1962) and Stokes et al (1962), found that ^{131}I Hypaque was only slightly bound to the serum proteins (5—10 per cent) in dogs and in several other species including human beings (Lasser et al 1962 and Kimbel 1963)

E *Studies on radioisotope renograms*

The external renal measuring technique with gamma sensitive scintillation detectors after administration of tracer doses of ^{131}I labelled substances made it possible to determine the isotope at a distance (Taplin et al 1956) The novelty of this technique was that it provided a possibility to appraise the function of each kidney separately and to demonstrate any disparity between them The time radioactivity curves obtained with such a technique consist of the following 3 segments (a) an initial short upswing immediately after the injection of the isotope and considered to be a measure of the initial passage of the radioactivity through the vascular bed of the kidney and the surrounding tissues (so called vascular segment) (b) a second segment with a less marked ascent towards a maximum value of the curve This segment was interpreted as representing the rate of active tubular cell uptake and passage of the substance through the tubuli (so called uptake segment) (c) a third segment following the maximum value was characterized by a precipitous fall in radioactivity (so called excretion segment) This segment was considered to express the urinary excretion and disappearance of the radioactivity from the area seen by the scintillation detector

Taplin et al (1956) reported that the test was mainly a qualitative test for the evaluation of differences in function between the kidneys They

CHAPTER III

PURPOSE AND PLAN OF THE INVESTIGATION

The purpose of the present investigation was to elucidate the distribution, renal and extra renal excretion of radioactive Hypaque, and the mechanism of the renal excretion of the substance its suitability for external measurement of the radioactivity over the chest and kidneys and to compare the clearance after administration of the substance by intravenous infusion with that noted after a single injection The purpose of this comparison was to find the most suitable clearance with the *single injection technique* as a measure of total renal function which might supplement external renal measurements with evaluation of the function of each kidney separately

Though it is generally agreed that ^{131}I Hypaque is eliminated mainly by glomerular filtration it is not certain whether tubular secretion and reabsorption of the substance occurs in human beings This is an important question especially concerning the possibility of using ^{131}I Hypaque instead of inulin as a measure of glomerular filtration One of the purposes of the present investigation was therefore to clear up the question whether tubular secretion plays any role in the clearance of ^{131}I Hypaque. In the *elucidation of this problem clearance* was studied after simultaneous administration of inulin PAH and ^{131}I Hypaque with and without blocking of the tubules by Probenecid.

The introduction of radioisotope renography made it possible to study the function of each kidney separately In the estimation of the clinical value of renography two problems presented themselves, *viz* the choice of radioactive substance and quantitative evaluation of the external renal curves

The purposes of this part of the investigation were 1) to find a suitable method for evaluating the renogram 2) *to analyse renographic differences* between the two kidneys, 3) to analyse the renogram in different types of renal disease 4) to demonstrate any correlation between parameters calculated from the renogram and the external chest curve 5) to demonstrate any correlation between parameters calculated from the renogram and other renal function data with radioactive Hypaque (clearance with intravenous infusion technique renal excretion rate disappearance from blood curve) and other renal function tests (creatinine clearance serum creatinine N P N and maximal specific gravity) 6) to estimate the agreement between the renogram and the findings made at excretion urography laparotomy and post mortem examination 7) to assess the clinical value of renography

der (Frohlich et al 1959, Bodfish & Roberti 1960, Scheer & zum Winkel 1960, Säterborg 1960, Abt & Balkus 1961, Feine & Bauer 1961, Johnson et al 1961, Magnusson 1962 and others)

Though the substance is still given as a single injection, other methods using intravenous infusion have also been used (Lindell 1963) In experimental and clinical trials large doses of unlabelled PAH and Probenecid have been given before the isotope test to improve the diagnostic value of the radioisotope renogram (zum Winkel et al 1961 c, Wax & McDonald 1962)

The range of indications for renography has been widened considerably Radioisotope renography is gentle, it requires only little time, the dose of radioactivity is small and there are no contraindications (Winter 1963) While unilateral renal injury in hypertension has been the commonest indication for examination, the renogram has proved useful also in the investigation of urinary obstruction, in the differential diagnosis between renal and post renal anuria or oliguria and in the study of the course of functional disorders and the renal excretion after operations on renal arteries, pelvis and ureters It has also recently proved useful in the postoperative evaluation of renal transplants in man (Collins et al 1963, Loken et al 1964)

Most investigators have found the renogram valuable as a supplement to morphological and other functional methods (excretion urography, retro grade pyelography, renal angiography and selective clearance tests) Some investigators have, however, questioned the value of the renogram compared with that of excretion urography, for example (Dollery 1960, Poker et al 1960, Moses et al 1961) It is mainly the difficulty in interpreting the external renal curves in an objective and correct way that is responsible for the uncertainty and doubt regarding the value of the test Evaluation of the curves by visual inspection alone or from different types of parameters calculated from the renogram have given widely divergent results

¹³¹I labelled Hypaque dissolved in 1 5—4 7 ml sterile water Concentration 1 99—3 38 mg/ml with a specific *activity of 115—700 µCi/mg* The purity of the radioactive preparation was checked by paperchromatography and was stated to be not less than 95 per cent (Abbott Lab pers comm)

In order to keep the amounts of radioactive substances used for renography and for intravenous infusions as uniform as possible the stock solutions were diluted with physiologic saline A working solution (solution A) was obtained by adding 1 0 ml of 45 per cent non-radioactive Hypaque (~ 450 mg) and adjusting the volume to 100 ml with sterile physiologic saline The specific activity of Hypaque in this solution when used varied from 0 9 µCi/mg to 3 µCi/mg

Hypaque solution B was prepared in the same way but with no addition of carrier Specific activity of Hypaque in this solution when used varied from 25 µCi/mg to 400 µCi/mg

The solutions were kept in glass bottles sealed with rubber membranes and stored up to 18 days in the dark at room temperature Solutions not used *within this time* were discarded

Purity and stability of ¹³¹I Hypaque solutions

All Hypaque solutions used by the author in clearance or renogram studies were routinely checked before and during use in the patients for *any free* I by measurement of the 24 hour thyroid uptake and by supplementary radiochemical analysis of randomly selected samples with high voltage paper electrophoresis

On two occasions a significant 24 hour thyroid uptake was noted On one of the occasions this prompted a more comprehensive analysis with high voltage paper electrophoresis in order to follow the liberation of iodide or other components during storage of this Hypaque solution

Technique

The thyroid uptake was measured at 24 hours by the routine technique used in our isotope laboratory This technique is essentially in accordance with that recommended by IAEA (1960) A solution of ¹³¹I Hypaque taken from the stock solution was diluted to a volume of 100 ml This solution was used as a standard in the calculation of thyroid uptake

High voltage paper electrophoresis was performed according to Wieland & Pfleiderer (1955) Paper Whatman filter paper No 3 MM The buffer used was pyridine glacial acetic acid water (100 10 890) pH 6 0 Voltage about 1800 volts and potential gradient about 45 volt/cm *Temperature* — 8° to — 10° C. Application 0 005—0 010 ml 8 cm from the cathode *Running time 60 minutes* The mobility of Hypaque was about 3 cm/1800 V/hour *and that of iodide* 18 cm/1800 V/hour *After separation* the papers were dried at +60° *for* 15 minutes The iodine

CHAPTER IV

RADIOCHEMICAL ANALYSIS OF 131I-LABELLED HYPAQUE

General remarks

Chemically sodium diatrizoate is 3,5-diacetamido 2,4,6 triiodo benzoic acid sodium salt ($C_{11}H_8I_3N_2NaO_4$) and its molecular weight is 635 9 Its structural formula is shown in Fig 1 Hypaque is a white crystalline solid, which *contains 59 87 per cent iodine* The iodine exists in a stable, organically bound state (Langecker et al 1954, Knoefel & Huang 1956, McChesney & Hoppe 1957) The Hypaque salt is highly water soluble Isolation and chemical analysis indicate that Hypaque is excreted unchanged in the urine (Langecker et al 1954, McChesney & *Hoppe 1957) Hypaque is synthetized* and supplied by Winthrop Stearns Inc, New York, U S A, in solutions of varying concentration for use in diagnostic radiology, especially of the kidneys The methylglucamine salt of diatrizoate (Renografin) has also been synthetized and is used as a contrast medium A mixture of sodium and methylglucamine salt of diatrizoate in the proportion 10 66 is marketed as Urografin

The non radioactive Hypaque (Hypaque 45 or 50 per cent) is a highly *soluble white crystalline solid* Hypaque is relatively thermostable and tolerates autoclaving Edathamilcalcium disodium 1 10,000 has been added as a stabilizer The solution should be *protected from strong light* Samples protected from light still comply with the control specification after six years' storage at room temperature Check examination with paper chromatography and thin layer chromatography have not revealed any impurities (Winthrop Stearns Inc pers comm)

131I-labelled Hypaque

The radioactive Hypaque was supplied by *Abbott Lab, Oak Ridge* Tenn, U S A It was supplied as a sterile and pyrogen free solution in rubber stoppered glass vials Each batch (stock solution) contained 2 mCi

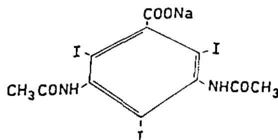


FIGURE 1 Sodium 3,5 diacetamido 2 4 6 tri iodobenzoate

The chemical identity of the two components

Radioactive and non-radioactive Hypaque were studied with high voltage paper electrophoresis to find out whether they behaved in the same way. The blackening of the autoradiograms in experiments using radioactive Hypaque coincided with the blue spots containing the non-radioactive Hypaque.

Since radioactive Hypaque was prepared by an exchange reaction between non-radioactive Hypaque and Na ^{131}I it was thought that some radioactive iodide might persist in the radioactive Hypaque solution. When Na ^{131}I was examined separately the radioactivity appeared as a single spot and it corresponded to the spot in the investigation of the Hypaque solution. Also the spot in autoradiograms of radioactive iodide corresponded to the spot in the investigation of the radioactive Hypaque solution. Thus the behavior of ^{131}I was similar to the corresponding component in the separation systems and autoradiograms of radioactive Hypaque solutions investigated.

Changes during storage of the solution

The changes in the composition of the preparation of radioactive Hypaque that had given a thyroid uptake of 7 - 8 per cent were followed by examining samples at different intervals. The results showed a further decrease of the Hypaque fraction (3 - 4 per cent) during 18 days storage and an almost corresponding simultaneous increase of the relative content of ^{131}I. No new fractions were detected.

The reproducibility of the separation method was investigated in 6 estimations of the above mentioned solution. Mean value and standard deviation of the content of ^{131}I Hypaque in per cent of the total radioactivity: *82.9 ± 0.5*.

Comments

The 24 hour thyroid uptake in clearance and renographic studies with different solutions showed that only on two occasions (all together three patients) did the thyroid uptake exceed 1.7 per cent indicating a higher degree of free iodide in the solution. No difference in uptake was noted between pure tracer solutions and solutions with addition of a small amount of non-radioactive Hypaque. There was good agreement between the thyroid uptake values and the corresponding results obtained with high voltage electrophoresis. In the two preparations where the uptake was increased the occurrence of free iodide was verified.

The behaviour of ^{131}I was similar to that of the main impurity component found in the radioactive Hypaque solutions. It was thus very probable that this fraction was identical with ^{131}I. High voltage electrophoresis showed a decrease of the radioactive Hypaque by a further 3—4 per cent during 18 days storage.

The results obtained with various radioactive Hypaque solutions were in good agreement with the 24 hour thy

containing compounds were developed according to Gmelin & Virtanen (1959) The localization of the radioactivity was determined by beta-ray scanning with an automatic windowless paper chromatogram scanner and by autoradiography with Gevaert Rapid x ray films and an exposure time of 1—2 days

Solutions

(1) ^{131}I Hypaque solutions A and B (see page 17)

(2) a water solution of carrier free Na ^{131}I with about the same radioactivity as solution B

(3) non radioactive Hypaque (45 per cent Hypaque solution) was diluted with physiologic saline to a concentration of 0 450 mg/ml

Results

24 hour thyroid uptake

In the amounts of radioactive Hypaque (25—50 μCi) given in both the clearance and renographic studies the dose gave between 60 000 and 120 000 cpm when measured with the apparatus and technique used The 24 hour thyroid uptake varied between 500—1000 cpm which corresponds to an uptake of about 0 4—1 7 per cent The thyroid took up an equal amount of the two solutions (A and B) used The thyroid uptake of two preparations exceeded the above limits (5 and 7—8 per cent respectively) These two preparations were therefore not used further The high values found for free iodide in these preparations were confirmed by high voltage paper electrophoresis

High voltage paper electrophoresis

Fig 2 shows the results of analysis with beta scanning of an electropherogram and an autoradiogram of a solution which had given a 24 hour uptake of 1 0 per cent in a patient As can be seen, a good separation was obtained with this electrophoretic technique The radioactive components are Hypaque (96 6 per cent), iodide (3 0 per cent) and unidentified impurity (0 4 per cent) Thus the two main components represented almost the entire activity, but some other irregular components sometimes occurred They were, however, never found to exceed 1 0 per cent The autoradiogram verified the existence of the two components in the electrophoretic paper studied The solutions which had given 24 hour thyroid uptakes of 5 and 7—8 per cent in patients, contained 10 and 16—17 per cent free radioactive iodide The corresponding values of radioactive Hypaque were 90 and 83—84 per cent in analysis with high voltage paper electrophoresis

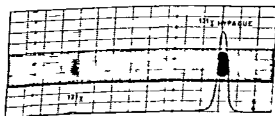


FIGURE 2 High voltage paper electrophoresis of fresh stock solution of radioactive Hypaque Autoradiogram and scan curve of the paper strip S Starting point

METHODS

Determination of radioactive amounts given

The following procedure was used for estimating the radioactivity of the amount given. Different amounts of radioactive Hypaque solution (0.2—0.8 ml) were drawn up in 2.0 tuberculin syringes. The syringe was placed and counted in the symmetry line of a scintillation detector with a collimator diameter of 65 mm. The distance between the crystal and the aperture of the collimator was 110 mm and that between the aperture and the syringe 140 mm. The content was then ejected into a volumetric flask. The volume was made up to 1000 ml with water and 2.0 ml was taken for counting in a well type scintillation detector. A good linear relation was found between the two counting series and a factor for converting count rate of the syringe to the count rate of the well type detector could be calculated.

Standards

0.10 ml of the stock solution of ^{131}I labelled Hypaque was removed with a syringe and its radioactivity measured under the scintillation detector. The content was ejected into a glass flask containing 1000 ml of water. 2.0 ml of this solution was counted daily in the well scintillation counter. This solution was used as a standard in the examination of blood, urine and bile samples.

Measurement of activity in blood, urine and bile samples

The samples were counted in a well scintillation detector with a 2" × 1 7/8" thallium activated iodide crystal and a 1 1/2" × 5/8" central well capable of receiving a tube containing a 5 ml sample. The detector was mounted in a lead shield and connected to a scaler. The capacity of the recording system of the scaler was tested by measuring radioactivities of varying strength. A linear relationship was found between amount of radioactivity and the pulse rate up to 100 000 cpm. The background was always measured before and after the series of sample were studied. The blood and bile samples were counted for 30 minutes and the urine samples for 10 minutes or as the time for a predeter

roid uptake found in patients in a previous study (Denneberg et al 1961 a), where the uptake was less than 1.0 per cent, and in animal studies using autoradiography (Denneberg et al 1960).

Blaufox et al (1963 d) found 5 per cent free iodide and about 5 per cent unidentified impurities in commercially available radioactive Hypaque. They thought these impurities to be at least partly responsible for the difference found between Hypaque and creatinine clearance in dogs. In animal experiments Kimbel & Borner (1955) found no significant radioactivity over the thyroid gland of the rat 24 hours after injection of ^{131}I Urografin, while zum Winkel & DeMaria (1961) reported an uptake of 0.41—0.47 per cent by the thyroid gland after 200 minutes. The importance of checking the purity of the radioactive solution is discussed in Chapter VII F.

METHODS

Determination of radioactive amounts given

The following procedure was used for estimating the radioactivity of the amount given. Different amounts of radioactive Hypaque solution (0.2—0.8 ml) were drawn up in 20 tuberculin syringes. The syringe was placed and counted in the symmetry line of a scintillation detector with a collimator diameter of 63 mm. The distance between the crystal and the aperture of the collimator was 110 mm and that between the aperture and the syringe 140 mm. The content was then ejected into a volumetric flask. The volume was made up to 1000 ml with water and 2.0 ml was taken for counting in a well type scintillation detector. A good linear relation was found between the two counting series and a factor for converting count rate of the syringe to the count rate of the well type detector could be calculated.

Standards

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mined number of counts (100,000). The standard deviation of the blood, bile and urine samples counts was always less than 1.5 per cent. The stability of the apparatus was checked daily by measuring the activity of a Na standard.

Chemical methods

Para aminohippuric acid (PAH) was determined largely by the method of Brun (1951). 1 g of p dimethylamino benzaldehyde, 50 ml of acetic acid, and 50 ml of physiologic saline were mixed. 5 ml of this reagent was added to 0.100 ml of native serum. Suitable dilutions of the urine were prepared before analysis. A 24 hour urine sample was diluted 1:50—1:200 and 0.100 ml of the diluted urine was taken. The reaction was read either immediately or several hours later at a wave length of 465 nm in a Zeiss PM Q II spectrophotometer against blanks taken before infusion of PAH. Standard solutions of PAH of 4 mg/100 ml were run in parallel. The reaction was carried out at room temperature. The error of a single determination (as calculated from 36 double determinations) was 1.06 per cent.

Inulin was determined by the method of Heyrowsky (1956) with slight modifications (Denneberg et al. 1961 a). The error of a single determination (36 double determinations) was 1.57 per cent.

DISTRIBUTION AND ELIMINATION OF ^{131}I LABELLED HYPAQUE

Introduction

The animal experiments showed a rapid elimination of Hypaque or Urografin from the blood by the kidneys and no substantial uptake in other organs and no signs of any significant extrarenal excretion.

Knowledge of the kinetics of Hypaque is necessary for evaluating the suitability of the substance for clearance tests with the single injection technique. This chapter is concerned with the blood concentration curves, the excretion of the test substance in the urine and bile as well as the distribution volume of ^{131}I Hypaque in a clinical series. The formulae given in the appendix for three different types of clearance tests with the single injection technique have been applied in the clinical series and comparisons have been made between the results obtained by the single injection and intravenous infusion technique.

A Material

Clearance studies were performed on 31 hospital patients including 18 with and 13 without diseases related to the kidneys. These patients included 21 in whom clearance was also studied by the intravenous infusion technique and the isotope renogram (Chapters VII and VIII). The initials, sex, age, body surface, haematocrit, plasma volume and the clinical diagnoses are given in Table I. The purpose of the investigation was not to assess the normal limits of the Hypaque clearance with a single injection of Hypaque but to compare the values obtained by different types of clearance tests in patients with and without impaired renal function. In two patients who had recently been subjected to choledocholithotomy biliary excretion was studied in bile collected from indwelling T tubes. In addition in 17 patients with and 13 without renal disease the excretion of the test substance in the urine was determined and separate analyses were made of venous blood. These patients are also included in the material examined with the isotope renograms (Chapter VIII).

B Technique

The patients were instructed to drink as much as they could before the test. They were examined in the semirecumbent position.

Table I. Clinical material (31 cases) used in study of clearance of Hypaque with single injection technique

Case	Sex	Age (years)	Height cm	Weight kg	BSA m²	Hcr %	PV ml	Diagnosis
FN	M	57	174	87	2.0	37	3760	Cephalalgia
IH	M	49	172	55	1.7	39	3040	Lumbar disc degen
SP	M	41	172	74	1.9	40	3430	Rheum arthrit
KA	M	32	183	70	1.9	45	3610	Epilepsy
DA	M	60	171	86	2.0	41	3790	Humeroscapular periarteritis
WJ	M	53	174	83	2.0	44	3520	Spondylosis deformans
LL	F	50	159	44	1.4	37	2150	Cholelithiasis
HB	F	33	155	48	1.4	40	2350	Epilepsy
AJ	M	55	170	71	1.8	45	3040	Neurasthenia
LK	M	22	184	72	1.9	39	3470	Rheum arthrit
SN	M	61	170	76	1.9	46	2960	Rheum arthrit
SA	M	42	167	65	1.7	44	2690	Neurasthenia
HM	F	54	161	61	1.7	37	2540	Cephalalgia
ML	M	22	185	80	2.1	43	3490	Nephropathy (Haematuria)
AO	M	53	174	73	1.9	36	3630	Proteinuria + Haematuria
RA	M	59	185	78	2.0	37	2500	Polycythemia vera + Nephrolithiasis
BL	F	34	164	55	1.6	35	2670	Ess hypertension
BA	M	52	173	75	1.9	46	3120	Nephropathy (Proteinuria)
GA	M	49	170	65	1.8	39	3170	Prostatic hypertrophy
HJ	M	29	196	100	2.3	40	4220	Nephropathy (Chron glom nephritis?)
HG	F	55	160	61	1.6	36	2470	Nephropathy (Proteinuria)
YS	M	49	174	73	1.9	47	3040	Ess hypertension
WA	F	64	161	48	1.5	37	2480	After leftsided nephrectomy + Diab mell
IB	F	54	157	76	1.8	40	2510	Nephropathy
AA	M	60	183	65	1.9	34	3690	Horseshoe kidneys
IG	F	30	162	66	1.7	38	2590	Ess hypertension
KK	F	29	168	54	1.6	42	2100	Bilat ren malformation
AO	M	55	180	66	1.8	26	4050	Chron pyelonephritis Nephrolithiasis
LV	M	34	168	65	1.7	27	4720	Nephropathy (Abuse of phenacetin)
TB	M	65	173	56	1.7	23	3780	Chron pyelonephritis
BF	M	37	164	62	1.7	23	3800	Chron glom nephritis

Injection of ¹³¹I-Hypaque. 0.2–1.0 ml (0.4 μCi/kg bodyweight) of solution A was given i.v.

Sampling and methods

Blood. With a catheter inserted in the left brachial artery samples were collected in 9 heparinized tubes (5 ml) in the course of 5—120 minutes. After haemolysis 2 ml was taken for counting. All measurements of the whole blood were corrected with 2 per cent for uptake of radioactive Hypaque by the red blood cells (Denneberg et al. 1961 a) which means that the activity

measured was calculated as the plasma concentration of ^{131}I Hypaque.

In the series of 28 other patients where the venous blood was analysed these samples were collected 60 and 90 minutes after the injection.

Urine. The patient voided urine before and 120 minutes after the injection. As a rule the urine voided during the rest of the day (24 hours) was collected (Table X). The 2 hour urine sample was diluted 1:10 and 2 ml was used for counting. 24 hour samples were used undiluted.

Bile was collected from the T tube during the 0–30, 30–60 and 60 minute–24 hour periods after the injection. Undiluted samples of 2 ml each were used for counting.

Haematocrit. In the analysis an 80 mm long tube was centrifuged for 15 minutes (3,000 rpm) in a centrifuge of type MSE haematocrit.

Plasma volume. The plasma volume was determined from sex, height *and weight with the aid of Gibson's* nomogram (Gibson et al. 1937). In 3 cases it was determined directly with Evans blue (v. Porat 1951).

The technique used for the studies of clearance with intravenous infusion technique is described in Chapter VII.

Calculations

The appendix (page 97) summarizes the kinetic problems associated with the single injection technique as well as the analysis of the clearance of substances eliminated entirely or partly by the kidneys. The general formulae are also given for the calculation of total plasma and renal clearance (with and without correction for renal dead space delay) *and of the distribution* volume.

The initial blood activity (cpm/ml) was *calculated from the amount of* radioactivity injected (cpm) and total blood volume. All the subsequent values *found were expressed* as a fraction of this and shows the proportion of the amount of radioactivity still in *the circulating blood*.

Slope analysis of the curve on semi-logarithmic paper was started from *the end of the curve according to the* standard procedure (Riggs 1963, page 146). As a rule 3 or 4 exponential functions of time were obtained. The intercepts and slopes (calculated according to the formula b = ln 2/T 1/2, where b = slope and T 1/2 = half time) were read and the total area was calculated according to the principles described in the appendix.

For the 2 hour renal clearance the correction *for the renal dead* space delay was calculated from the urine flow, after which the corresponding area under the plasma curve was determined by numerical or graphical integration (see appendix). The urine values (cpm/ml) *from 2 and 24 hour* tests respectively are expressed as a fraction of the amount of radioactivity injected. *The total clearance* was calculated according to formula No. 1, *the 24 hour renal clearance* according to No. 2 and *the 2 hour renal clearance* according to No. 3. *The distribution volume* was calculated according to formula No. 6 with the aid of the intercepts and the slopes.

Calculation of the clearance by the constant intravenous infusion technique is given in Chapter VII.

The clearance values used in this chapter were not corrected to hold for 1.73 m^2 body surface.

Retention per cent. The retention of ^{131}I-Hypaque in the blood was calculated from both the arterial and the venous curves. This parameter consists of the quotient between the 90 minute value and the 60 minute value × 100. The retention per cent is compared with other renal function data for ^{131}I Hypaque (clearance respectively urinary recovery) in this chapter (see below) and in Chapter VIII it is given in relation to parameters calculated from the external chest and renal curves.

C. Results

Blood curve. Fig. 3 a gives a scatter diagram of the blood values in the 13 controls with the mean concentration curve (broken). The curve falls first steeply and then gently. After 5 minutes the curve has fallen to 35 per cent, after 10 minutes, to 25 per cent, after 1 hour to 11 per cent and after 2 hours to 7 per cent. The individual curves are still slightly concave in the semilogarithmic scale. Table IX shows the calculated intercepts and exponential constants for the 13 controls.

Fig. 3 b shows the blood curves for the 18 patients with renal disease. For natural reasons the individual curves differed in shape. The series also includes normal curves. This is because the series consisted of patients with signs of renal disease but without manifest impairment of renal function. At 5 minutes the values lay between 25 and 52 per cent, and at 10 minutes, between 20 and 42 per cent. The slope successively became more gentle in patients with impaired renal function. One hour after the injection the values were between 9 and 26 per cent, and after 2 hours between 5 and 22 per cent. Neither in this group were the individual curves linear at 120 minutes. Table IX gives the intercepts and exponential constants for these 18 cases.

Excretion in urine. The excretion values noted at 2 hours in the 3 controls were 51, 54 and 62 per cent (mean value 56 per cent). The same mean value was obtained for 8 patients with a clearance of more than 100 ml/min in the infusion clearance test. At 24 hours 95 per cent (observed range 86—101 per cent) had been excreted by 23 controls. This figure may be somewhat too low owing to incomplete collection of voided urine. One control excreted 2 per cent and another 3 per cent during the second day. In the patients with renal diseases the values were lower, although in some of them the urinary recovery was high. The results of urinary recovery (2 and 24 hours) are given in graphical and tabular form (Figs. 6 and 7, Table X).

Excretion in the bile. In the two controls where the excretion of the bile was followed after a single injection of ^{131}I Hypaque the 30 minute, 60 minute and 24 hour values noted

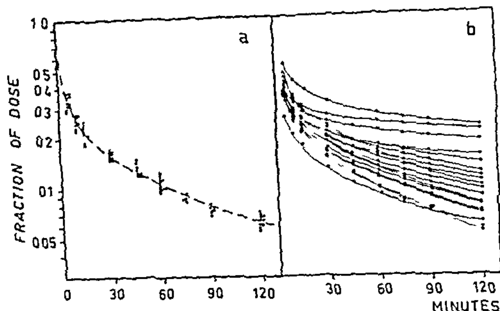


FIGURE 3a. Blood concentration of Hypaque in 13 controls expressed as fraction of dose given and mean concentration curve (broken). b. Curves for blood concentration of Hypaque in 18 cases with renal disease.

were 0.01, 0.03, 0.13 per cent in one and 0.03, 0.04 and 0.22 per cent in the other.

Clearance correlations

Table X gives the clearance with the intravenous infusion technique and the total 2- and 24-hour renal clearance, and in Figs. 4 a, b and c these three clearances are plotted against the clearance with infusion technique. The figures show a good correlation between clearance with the infusion technique and those with single injection technique (r = + 0.95, + 0.98 respectively 0.97). The total clearance, however, differs markedly from the other two in that the majority of the values are above the 45° line. The calculated regression line confirms that the values for the total clearance are systematically higher and above the 45° line: $y = 0.936\ x + 21.3$ (where y = total clearance and x = clearance with the infusion technique). Also the 24-hour renal clearance showed a similar picture with most of the cases above the 45° line, but this tendency was much less marked than that noted for the total clearance. The regression line was $y = 1.009\ x + 3.6$, where y = 24-hour renal clearance. The smallest deviation was shown by the *2-hour renal* clearance: the values were crowded around the 45° line and the regression line ($y = 0.972\ x + 0.7$, where y = 2-hour renal clearance) deviated only slightly from the identity line.

Calculation of the clearance by the constant intravenous infusion technique is given in Chapter VII

The clearance values used in this chapter were not corrected to hold for 1 73 m² body surface

Retention per cent The retention of ^{131}I Hypaque in the blood was calculated from both the arterial and the venous curves This parameter consists of the quotient between the 90 minute value and the 60 minute value × 100 The retention per cent is compared with other renal function data for ^{131}I Hypaque (clearance respective ly urinary recovery) in this chapter (see below) and in Chapter VIII it is given in relation to parameters calculated from the external chest and renal curves

C Results

Blood curve Fig 3 a gives a scatter diagram of the blood values in the 13 controls with the mean concentration curve (broken) The curve falls first steeply and then gently After 5 minutes the curve has fallen to 35 per cent after 10 minutes to 25 per cent after 1 hour to 11 per cent and after 2 hours to 7 per cent The individual curves are still slightly concave in the semilogarithmic scale Table IX shows the calculated intercepts and exponential constants for the 13 controls

Fig 3 b shows the blood curves for the 18 patients with renal disease For natural reasons the individual curves differed in shape The series also includes normal curves This is because the series consisted of patients with signs of renal disease but without manifest impairment of renal function At 5 minutes the values lay between 25 and 52 per cent, and at 10 minutes between 20 and 42 per cent The slope successively became more gentle in patients with impaired renal function One hour after the injection the values were between 9 and 26 per cent and after 2 hours, between 5 and 22 per cent Neither in this group were the individual curves linear at 120 minutes Table IX gives the intercepts and exponential constants for these 18 cases

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Excretion in the bile In the two controls where the excretion of the bile was followed after a single injection of ^{131}I Hypaque the 30 minute 60 minute and 24 hour values noted

the blood within 2 hours The initial segment falls rapidly also in cases with renal insufficiency but the later decline of the curve is much slower than in the controls This is apparent on visual examination as well as on comparison between the exponential constants of the curves

The rapid fall in the blood concentration was in good accord with that found by Schlungbaum & Billion (1956 b) with ^{131}I labelled Urografin and by Bruncht (1961) Denneberg et al (1961 a) and Stokes & Ter-Pogossian (1964) with ^{131}I labelled Hypaque The rapid elimination of the substance from the blood corresponds to the rapid excretion in the urine with more than 50 per cent in 2 hours, 95 per cent in 24 hours and 97—98 per cent in 48 hours These values agree with those found by Schlungbaum & Billion (1956 b) who reported 60 per cent in 3 hours and 90 per cent in 24 hours Similar results have been published by Kimbel & Borner (1955) and Langecker et al (1954) who found 24—54 per cent in 1 hour and 27—44 per cent in 2 hours while their 24 hour values were much lower (54—65 per cent) No explanation was however offered for the low level of these values It is clear from our results that practically all ^{131}I Hypaque is eliminated by the kidneys in other words

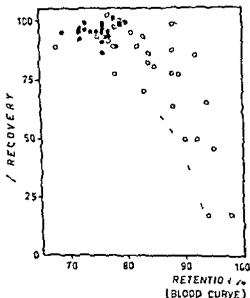


FIGURE 6 Relationship between 24 hour urinary recovery and retention of Hypaque calculated from blood curve (53 cases) Demarcation lines of the field are based on observed values Solid circles Controls Open circles Cases with renal disease

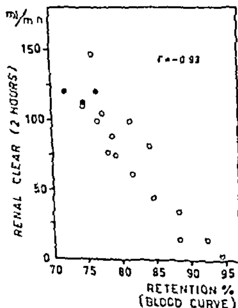


FIGURE 5 Relationship between 2 hour renal clearance and retention of Hypaque calculated from blood curve (18 cases) Solid circles Controls Open circles Cases with renal disease

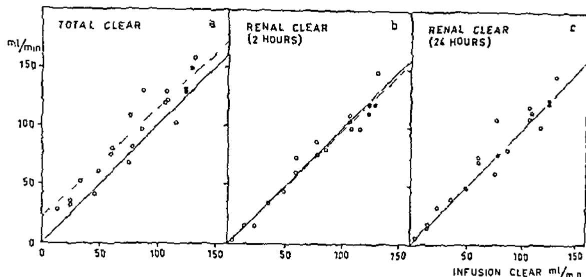


FIGURE 4 a Total clearance plotted against clearance of Hypaque with infusion technique (21 cases) b 2 hour renal clearance plotted against clearance of Hypaque with infusion technique (18 cases) c 24 hour clearance plotted against clearance of Hypaque with infusion technique (20 cases) Uninterrupted line 45° line Broken line Calculated regression line Solid circles Cases without renal disease Open circles Cases with renal disease The clearance values are not corrected to hold for 1 73 m body surface

Comparison between retention per cent and 2-hour renal clearance and urinary recovery

Fig 5 gives the 2 hour renal clearance plotted against retention per cent in 18 cases The figure shows a negative correlation between the values (r = — 0 93) *i e* the greater the retention the lower the clearance

The 24 hour urinary recovery is plotted against retention in 53 cases in Fig 6 which shows a non linear relation between these variables A similar relation was found between the urinary recovery (2 respectively 24 hours) and the 2 hour renal clearance in 25 and 18 cases, respectively (Fig 7) The figure shows that the 2 hour recovery per cent varies more closely with clearance than the 24 hour recovery, the latter recovery often being high in cases with impaired renal function

The limits given in Figs 6 and 7 are based on observed and not on calculated values

Distribution volumes

Table X gives the distribution volume for 31 cases The mean for the 13 controls was 13 4 litres with an observed range of 8 6 to 18 3 litres which corresponds to 20 respectively 13—28 per cent of bodyweight The 18 patients with renal disease had a mean value of 13 6 litres with an observed range of 10 6 to 18 6 litres and 20 and 15—28 per cent respectively of bodyweight

D Discussion

The results show that the normal plasma curve for ^{131}I Hypaque rapidly falls after a single injection and more than 90 per cent of the activity leaves

the blood within 2 hours The initial segment falls rapidly also in cases with renal insufficiency but the later decline of the curve is much slower than in the controls This is apparent on visual examination as well as on comparison between the exponential constants of the curves

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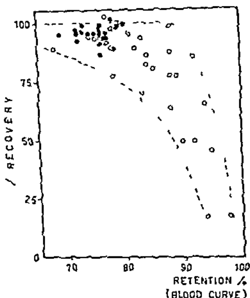


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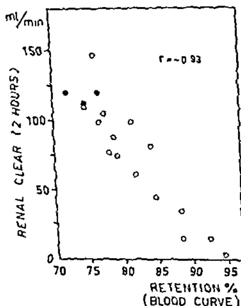


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than 50 per cent in 2 hours 95 per cent in 24 hours and 97—98 per cent in 48 hours These values agree with those found by Schlungbaum & Billion (1956 b) who reported 60 per cent in 3 hours and 90 per cent in 24 hours Similar results have been published by Kimbel & Börner (1955) and Langecker et al (1954) who found 24—54 per cent in 1 hour and 27—44 per cent in 2 hours while their 24 hour values were much lower (54—65 per cent) No explanation was however offered for the low level of these values It is clear from our results that practically all [131]I Hypaque is eliminated by the kidneys in other words,

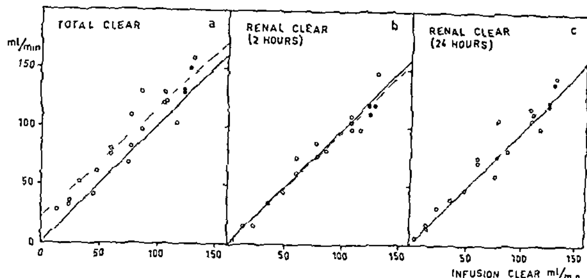


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D Discussion

The results show that the normal plasma curve for ^{131}I Hypaque rapidly falls after a single injection and more than 90 per cent of the activity leaves

and 23.0 per cent for both sexes together. Bianchi (1961) reported somewhat higher values for the distribution volume of ^{131}I Hypaque (25—35 per cent) but he gave no details about the method used. It is possible that he used the formula intercept/final slope which, according to Riggs (1963), gives artificially high values.

The retention per cent was calculated mainly as a simple measure of retention of Hypaque in the blood. Since the parameter is calculated as a quotient of the values obtained from two separate blood analyses, it avoids the otherwise necessary repeated sampling. The good relation with renal clearance, urinary recovery and retention showed that retention may be taken as a measure of the total renal function and consequently supplement other renal function data. In Chapter VIII retention calculated from the blood curve is compared with other parameters calculated from external chest and renal curves and thereby provides a possibility of studying the relation between external and internal isotope measurements.

On comparison between the clearance with the intravenous infusion technique and total, respectively 2 and 24 hour renal clearances, the 2 hour renal clearance was found to be the best of the three clearances with the single injection technique. The total clearance systematically showed artificially high values. This may be explained by the fact that the plasma curve had not reached its final slope because Hypaque had not yet attained its final distribution. This means that the area obtained was too small and the total clearance according to formula 1 was too high. To use the total clearance of Hypaque the final slope should therefore be calculated from a curve covering a longer period, e.g. 5 hours, in analogy with the studies of Hypaque distribution volume. On comparison between the 2 and 5 hour total clearance values in the abovementioned five cases the 5 hour values were 7—11 ml/min lower than those noted at 2 hours.

The calculation of the 2 hour renal clearance according to the formula 3 did not give too small an area under the curve because an observed limited part of the total area under the plasma curve was used. The factors which must be considered are the radioactivity in the renal dead space at the end of the period of urine collection and the magnitude of the urine flow during the clearance period. These factors and the corrections for transport and mixing delay, respectively, are discussed in the appendix (page 97). It is clear from Table X that in most of the patients urine flow was acceptable; only in 3 of the 18 was it below 1.0 ml/min. In 2 of these cases the correction for total delay was considerable (10 minutes) while the remaining had between 4.3—6.9 minutes.

The results show that the 2 hour renal clearance after a single injection can be used instead of the clearance with the infusion technique of ^{131}I Hypaque. It also appears that renal 24 hour clearance can be used, while the total clearance based on the values

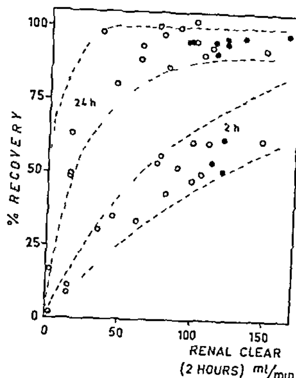


FIGURE 7. Relationship between 2 and 24 hour urinary recovery and 2 hour renal clearance of Hypaque (18 and 25 cases). Demarcation lines of the two fields are based on observed values. Solid circles: Controls. Open circles: Cases with renal disease.

extrarenal excretion is insignificant in normals.

The low values found for excretion of ^{131}I-Hypaque in the bile are in agreement with results obtained by Kimbel & Börner (1955), who found a small amount of radioactivity in the duodenal juice 40 minutes after the injection. They also demonstrated a slight activity in the gastric contents, but none in the saliva. As to the faecal excretion Schlungbaum & Billion (1956 b) found 1—2 per cent radioactive Urografin 48 hours after the injection. It should also be mentioned that the uptake by the thyroid (see Chapter IV) was low, usually below 1 per cent (0.4—1.7 per cent). These data show that ^{131}I Hypaque (or ^{131}I Urografin) is normally excreted almost quantitatively by the kidneys.

In the present investigation, partly for practical clinical reasons, the plasma curve was not followed for more than 120 minutes, which appeared to be sufficient. Earlier preliminary clearance studies have given good results within such an interval (Denneberg et al. 1961 a). Many substances require a much longer time for the plasma curve to reach its true final slope. If sampling is stopped before the curve has reached its final slope and if the slope of the last segment obtained is extrapolated to infinity, the total distribution volume calculated from formula 6 will be artificially small (see appendix). The shorter the period that the plasma curve is followed the larger the error. Examination of controls with Hypaque for 5 hours showed that the values for distribution volume calculated from this long curve were larger than the corresponding 2 hour values. Thus the value found for five normals was on the average 2.4 litres or 3.4 per cent higher for 5 hours than for 2 hours. The latter values are minimum values for the Hypaque distribution volume. The values obtained from the 5 hour curve imply that the distribution volume should be increased from 20 to 23.4 per cent of bodyweight which value is exactly the same as the bromide space. Moore et al. (1963) found the bromide space (after correction for uptake of bromide by red blood cells) to be 23.9 per cent of the bodyweight for males, 22.0 per cent for females.

THE RENAL EXCRETION MECHANISM OF ^{131}I-LABELLED HYPAQUE

Introduction

The investigations on animals and on humans have given divergent results regarding the renal excretion mechanism of Hypaque. The possibility of a partial tubular secretion and/or reabsorption could not be excluded. Since no experiments had been performed with inhibitors of tubular activity, the question was not definitely cleared up. To study the mechanism of renal excretion of Hypaque and especially the tubular secretion, clearance studies were performed with the infusion technique and simultaneous administration of inulin, PAH and radioactive Hypaque with and without blocking of the tubuli with Probenecid.

A Material

The material, which consisted of 60 patients, was divided into three series (A, B, C). The age distribution of the series are given in Table II.

Series A consisted of 29 patients with various diseases not related to the kidneys. Laboratory studies revealed no signs of renal disease in these patients. They were normotensive and afebrile. The initials, sex, age, height, weight, body surface area and diagnoses are given in Table III.

Series B consisted of 22 patients who had been admitted to the medical department on one or more occasions because of diseases affecting the kidneys or for investigation of suspected renal disease. Initials, sex, age, height, weight, body surface area and diagnoses are given in Table IV.

Series C consisted of 9 female patients including one with proteinuria (chronic glomerulonephritis?), 2 with essential hypertension and 6 with diseases unrelated to the kidney. The initials, sex, age, height, weight, body surface area and diagnoses are given in Table V. The influence of Probenecid was tested in this series.

Series A served as a control series in comparative clearance studies with various substances. Series B represented a group of patients with renal disease with varying degree of renal insufficiency. Series A and B were studied under identical conditions, while series C was examined under varying conditions after injection of Probenecid.

Series A was divided into four subgroups according to the composition of the infusion solution. A 1: the first 18 patients in Table II. A 2: the following three patients (G J, R E, M N). A 3: four patients (U S, V H, A D, I S). A 4: four patients (M P, A P, G S, C J).

from a 120 minute curve did not give correct clearance. On the other hand the total clearance calculated from a plasma curve followed for a long time seems to provide a possible method but from a clinical point of view such a long examination period is disadvantageous. The advantage of total clearance is that the determination is based entirely on the plasma values. No collection of urine is necessary and all risks of errors due to incomplete collection are thereby avoided. Single injection clearance has several advantages over clearance with the intravenous infusion technique: it requires no infusion aggregate, it requires no equilibration period and enables simultaneous evaluation of the total renal function and renography.

The greatest disadvantage from a practical clinical point of view is, however, the necessity of arterial sampling. This has been considered necessary because of the arteriovenous difference in the concentration of the substance with falling plasma values after a single injection (Brun et al. 1949 b, Smith 1951). As is apparent from the appendix (page 97), it is probable that the formulae given (1 and 3) can be used for venous blood if the blood curves are followed for a long time, e. g., one day. An alternative to arterial sampling would be the use of capillary samples, but then the amount of ^{131}I labelled Hypaque injected would have to be increased.

Finally, it should be emphasized that the single injection clearance can only be used for substances whose clearance rate is independent of the plasma concentration. Thus when Diodrast is used, the proportion taken up by the red blood cells and that bound by protein result in markedly falling clearance values at low plasma concentration (Hilden 1946, Hogeman 1948, Newman et al. 1949, Block & Burrows 1960). As far as Hypaque is concerned the uptake by the red blood cells is small (Denneberg et al. 1961 a), the protein binding is insignificant (Lasser et al. 1962) and the results of clearance with the intravenous infusion technique suggest no variation of clearance with the plasma level (Chapter VII).

THE RENAL EXCRETION MECHANISM OF 131I-LABELLED HYPAQUE

Introduction

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Table II Survey of material used in investigation of renal excretion mechanism of Hypaque

Series	No of cases			Age (years)	
	Total	Female	Male	Range	Mean
A	29	11	18	21—69	44.7
B	22	7	15	22—65	48.0
C	9	9		21—64	45.1
Total	60	27	33	21—69	46.0

Table III *Controls* used in study of clearance of Hypaque with intravenous infusion technique

Series	Case	Sex	Age (years)	Height cm	Weight kg	BSA m^2	Diagnosis
A 1	MA	F	41	157	93	1.9	Psychoneurosis
	UA	F	44	158	60	1.6	Neurasthenia
	LO	F	49	148	53	1.5	Rectal cancer
	DS	F	51	175	114	2.3	Gastric ulcer
	BJ	M	24	173	59	1.7	Rheum arthrit
	JL	M	24	178	79	2.0	Cephalgia
	AF	M	25	170	58	1.7	Tonsillitis
	SH	M	26	181	70	1.9	Angiolipoma of skin
	AB	M	41	179	75	1.9	Cephalalgia
	SP	M	41	172	74	1.9	Rheum arthrit
	IH	M	49	172	58	1.7	Lumbar disc degen
	SL	M	50	171	77	1.9	Rheum arthrit
	NS	M	51	179	87	2.1	Cerebral a—v aneurysm
	GZ	M	54	178	99	2.2	Epilepsy
	LN	M	57	174	87	2.0	Cephalalgia
	BK	M	61	169	70	1.8	Glaucoma
	NK	M	64	171	85	2.0	Neurasthenia
	JJ	M	65	173	62	1.7	Gastric ulcer
A 2	GJ	F	28	168	61	1.7	Psychoneurosis
	RE	F	33	165	44	1.5	Gastritis
	MN	F	38	165	66	1.7	Rheum arthrit
A 3	US	F	25	155	40	1.3	Depression
	AH	F	43	166	56	1.6	Cephalalgia
	AD	F	64	170	81	1.9	Angina pectoris
	ES	M	69	167	66	1.8	Pulm emphysema
A 4	MP	F	35	167	54	1.6	Psychoneurosis
	AP	M	43	168	53	1.6	Psychoneurosis
	GS	M	49	170	70	1.8	Psychoneurosis
	CJ	M	52	184	67	1.9	Cephalalgia

Table IV Some data about cases of renal disease used in study of clearance of Hypaque with intravenous infusion technique

Series	Case	Sex	Age (years)	Height cm	Weight kg	BSA m²	Diagnosis
B	ÖD	M	44	170	74	1.9	Myelopathy + Atonia vesic. urin
	BL	F	34	164	55	1.6	Ess hypertension
	JL	M	53	185	62	1.8	Rheum heart disease+Proteinuria
	ML	M	22	185	80	2.1	Nephropathy (Haematuria)
	AO	M	53	174	73	1.9	Proteinuria+Haematuria
	BA	M	52	173	78	1.9	Nephropathy (Proteinuria)
	JA	M	49	172	58	1.7	Prostatic hypertrophy
	RA	M	59	185	78	2.0	*Polycythemia vera+Nephrolithiasis*
	FN	M	61	175	87	2.0	Prostatic hypertrophy
	WA	F	64	161	48	1.5	After left sided nephrectomy + Diab *mell*
	GA	M	49	170	65	1.8	Prostatic hypertrophy
	HG	F	55	160	61	1.6	*Nephropathy (Proteinuria)*
	AS	M	49	174	73	1.9	Ess hypertension
	BH	F	56	161	60	1.6	Sclerodermia
	BJ	M	29	190	100	2.3	Nephropathy (Chron glom nephritis?)
	FB	F	54	157	76	1.8	Nephropathy
	AS	M	60	183	65	*1.9*	*Horseshoe kidneys*
	IC	F	30	162	66	1.7	Ess hypertension
	KK	F	29	168	54	1.6	Bilat ren malformation
	AÖ	M	55	180	76	1.8	Chron pyelonephritis+Nephrolithiasis
	TB	M	65	173	56	1.7	Chron pyelonephritis
	IN	M	34	168	65	1.7	Nephropathy (Abuse of phenacetin)

Table V Some data about cases used in study of effect of Probenecid

Series	Case	Sex	Age (years)	Height cm	Weight kg	BSA m²	Diagnosis
C	KC	F	21	156	60	1.6	Epilepsy
	BN	F	30	161	66	1.7	Proteinuria
	AI	F	31	168	73	1.8	Cephalalgia
	LN	F	40	170	68	1.8	Otitis externa
	LC	F	45	150	48	1.5	Rheum arthrit.
	SW	F	56	158	49	1.5	Rheum arthrit
	RI	F	63	161	72	1.8	Arthrosis deformans
	RR	F	64	160	71	1.8	*Ess hypertension*
	AI	F	56	164	82	1.9	*Ess hypertension*

B Technique

Substances

Pyrogen free inulin in sterile 50 ml ampoules containing a 10 per cent solution (Laevosan Gesellschaft, Germany) was used Para aminohippuric acid was supplied in sterile 50 ml ampoules containing a 20 per cent solution of the sodium salt (Sharp and Dohme U S A) Non radioactive Hypaque in a 45 per cent solution in sterile 20 ml ampoules was used (Winthrop, England) The radioactive solution used is described in Chapter IV

Composition of the infusion solution

The infusion solution contained about 0 75 per cent inulin 0 60 per cent para aminohippuric acid and 0 079 per cent non radioactive Hypaque in 600 ml saline

A varying amount of radioactive Hypaque (0 20—1 80 ml) from solution A (see Chapter IV) was added to the infusion solution This amount was adjusted to the weight of the subjects (0 6—1 2 μCi/kg bodyweight which corresponded to 30—80 μCi) Of the radioactive dose one fifth was given in the priming dose and four fifths in a drip solution This produced a plasma concentration of 1—4 mg PAH, 10—20 mg inulin and 1—2 mg Hypaque per 100 ml plasma

This infusion solution was given to all the patients but with the following exceptions and modifications

Series A 2 Three cases in this series were given radioactive Hypaque from the stock solution (see Chapter IV) without any addition of non radioactive Hypaque The concentration of non radioactive Hypaque in the infusion solution was about 0 0002 per cent and the calculated plasma concentration of the substance was extremely low (0 002—0 003 mg/100 ml plasma)

Series A 3 To the four cases in this series radioactive Hypaque was given from the stock solution (see Chapter IV) In addition non radioactive Hypaque was added in such quantities as to obtain a final concentration of 0 040 per cent non radioactive Hypaque in the infusion solution which produced a plasmaconcentration of 0 6—0 8 mg/100 ml

Series A 4 In the four cases in this series a method was used which was identical with conventional clearance tests The labelled material was added to sufficient 45 per cent stable Hypaque to maintain a constant blood level at, or just below, 6 mg/100 ml plasma during the clearance determination The concentration of non radioactive Hypaque in the infusion solution was 0 5 per cent

Series B In this series of patients with renal diseases the concentration of PAH inulin and non radioactive Hypaque was varied according to the impairment of renal function in those with renal insufficiency In 8 of 20 patients in this series no PAH was given in the infusion solution

The total amount of radioactive Hypaque given during the test (= radioactivity of Hypaque in the priming dose + radioactivity from the amount

included in the continuous infusion) varied between 30 and 50 µCi. The plasma concentration of PAH, inulin and non radioactive Hypaque in the pathological series (series B) was of the same order and within the same limits as in the control series (series A 1).

Examination procedure

The patients were in the fasting state but had been instructed to drink as much fluid as possible before the test to obtain a good urine flow. The test was performed in the morning and took 2—2 1/2 hours. During the infusion tests the patient was recumbent.

Apparatus and performance of infusion

The substances were administered in a priming dose followed by intravenous infusion at a constant rate. An infusion pump was used which permitted the administration of the solution at 4 ml/min in 10 minutes, after which the infusion was given by means of the drip method by which the rate was standardized (3—4 ml/min). The first clearance period was started about 40 minutes after the beginning of the drip infusion. Three 20—30 minute clearance periods were used (Fig. 8).

In the experiments with Probenecid in series C the examination period was somewhat longer than in series A because of supplementary clearance periods (2 1/2—3 hours). On the other hand the individual clearance periods were somewhat shorter (15—20 minutes). After 2 initial clearance periods Probenecid solution was given intravenously for 10—15 minutes. The solution was in sterile 100 ml ampoules of 1 per cent (Probenecid natrium solution)[1]. The doses given varied between 500 and 2200 mg. After the end of the Probenecid injection a new clearance period was started and 3 further periods were studied.

Sampling

Blood. Samples of blood (8—10 ml per sample) were drawn from an antecubital vein 5 respectively 15 *minutes after* the beginning of each clearance period.

Urine. The urine samples were collected with an *indwelling* catether. During urine collection periods the catheter was allowed to drain into a glass. About two minutes *before* the end of each period the bladder was emptied as completely as possible by suprapubic pressure. Residual urine was removed by instillation of 50 ml sterile saline. Sometimes after the urine had been expelled 50 ml of air was introduced into the bladder and expelled in order to remove the last washout fluid. The total volume of the fluid (= urine plus washout fluid) was measured.

Comments on clearance experiments

In one of the cases (B N) the patient reported nausea and diffuse abdominal pain and cold sweat during admini

[1] Probenecid solution was generously supplied by AB Astra.

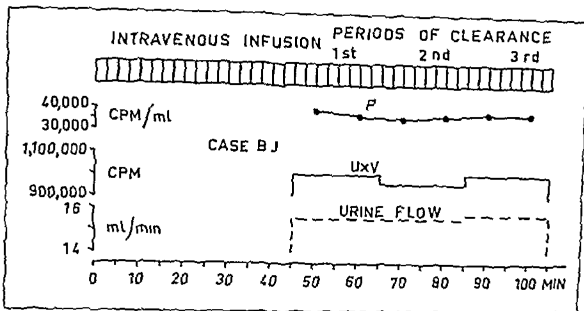


FIGURE 8 Clearance of Hypaque with intravenous infusion technique used and calculated clearance values in B J (control) P Plasma sample (cpm/ml) U Urine sample (cpm ml) V Urine volume (ml)

¹³¹I Hypaque clearance (ml/min)	135	134	135
Inulin clearance (ml/min)	116	126	117
PAH clearance (ml/min)	669	763	672
Quotient Hypaque/inulin clearance	1 16	1 06	1 15
Quotient Hypaque/PAH clearance	0 20	0 18	0 20
Filtration fraction (Inulin/PAH clearance × 100)	17	17	17

stration of Probenecid, and a moderate transient decrease in the blood pressure was noted But no severe signs of shock were registered In the remaining patients Probenecid produced no side effects

C Treatment of data

Clearance calculations

For each period 2 0 ml undiluted plasma, 2 0 ml undiluted urine (from the urine plus washout fluid) and 2 0 ml diluted standard (from the infusion solution) were counted (see Chapter V) The standard used was diluted 1 10 The plasma concentration of Hypaque was calculated by the formula $\frac{\text{serum sample cpm}}{\text{standard cpm}} \times$ concentration of Hypaque (mg/ml) in infusion solution ¹³¹I Hypaque clearance (C_{Hyp}) was calculated by inserting cpm/ml urine and plasma directly into clearance formula $C_{Hyp} = \frac{U \times V}{P}$, where U = cpm/ml of urine V = ml of urine per min and P = cpm/ml plasma The plasma level was calculated as the arithmetic mean of the two blood samples collected at the beginning and the end of each period

The clearance of para aminohippuric acid (C_{PAH}) and inulin (C_{In}) were calculated by the method

$$\frac{\text{urine volume (ml)} \times \text{urine ext} \times \text{dilution}}{\text{serum extinction}}$$

The clearance values were then corrected to hold for 1 73 m² body surface area

In the above mentioned clearance experiments the standard performance according to Smith (1951) with a priming dose with increased concentration of PAH was not used The main purpose of the examination was to study the excretion mechanism of Hypaque and to avoid the risk of any simultaneous competition effect between Hypaque and PAH the latter substance was given in such quantities that the plasma level was relatively low

After an equilibration period of 45—50 minutes the plasma level of the radioactive Hypaque in half of the cases was stable during the entire test period while in the other half (especially in cases with impaired renal function) the plasma level increased slowly by 10—12 per cent during the entire experimental period Thus implies an increase of about 3—4 per cent per period This increase was accompanied by a moderate increase in the secretion of urine The results were not corrected for increase in blood concentration or increase of the urine output Correction would be justified because rising plasma levels give increasing clearance (Brun et al 1949 b) and changes in delay time (Bojesen 1954 a) The reasons why no correction was made in the present investigation were 1) In the appraisal of a quotient between the clearances of the two substances which were determined simultaneously, the effect of the above changes in the blood concentration were insignificant 2) In these clearance experiments urine flow was good and correction for changes in delay time was therefore of less importance

D Results

¹³¹I Hypaque clearance

Tables XI XII and XIII give the initials of the subjects in series A and B the haematocrit number and duration of clearance period urine flow, clearance of Hypaque inulin PAH quotient Hypaque/inulin clearance, filtration fraction × 100 and the mean of the 3 clearance periods and of the quotients as well as the means after correction to hold for 1 73 m² body surface area

The mean value for Hypaque clearance in the entire control series (A) was 115 ml/min with an observed range of 80—153 ml/min The mean for the females was 116 ml/min and for the males 114 ml/min In the 3 series (A 2 3 4) where different doses of non radioactive Hypaque were added to the radioactive solution the values were of the same order as in the larger series (A 1)

In the series with renal diseases (B) the clearance was 15—117 ml/min That clearance values fell within the normal range in this pathological series may be explained by the fact that it included cases with clinical signs of renal disease but without

manifest impairment of renal function

Inulin and PAH clearance

In the control series (A 1) the mean inulin clearance was 102 ml/min with an observed range of 71—130 ml/min and that of the PAH clearance 498 ml/min with a range of 316—708 ml/min (in 3 cases the inulin clearances were not calculated for technical reasons and in 2 cases the PAH clearance was not studied)

In series B the clearance of inulin and PAH in cases with renal disease sometimes fell within the same range as in the controls The inulin values ranged from 13 to 113, and those for PAH from 32 to 620 ml/min (in 8 of the cases PAH clearance was not studied)

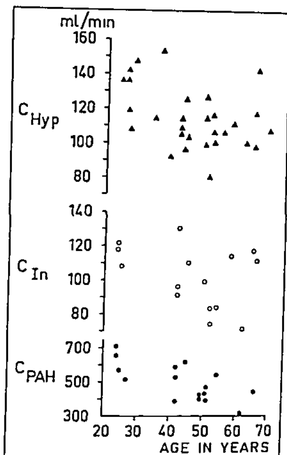


FIGURE 9 Mean clearance of Hypaque inulin and PAH in relation to age in controls (Series A 1) C_{Hyp} Hypaque clearance C_{In} inulin clearance C_{PAH} PAH clearance The clearance values are corrected to hold for 1 73 m² body surface

Age distribution

To study the effect of age on renal function the material (series A) was divided into 3 groups 20—39, 40—49, 50—69 years The mean values found for ^{131}I Hypaque clearance were 127 ml/min (9 patients) in the 1st age group, 110 ml/min (9 patients) in the 2nd group and 108 ml/min (11 patients) in the 3rd group The corresponding values for inulin were 116 ml/min (3 patients), 105 ml/min (5 patients) and 93 ml/min (7 patients) and for PAH 610 ml/min (4 patients) 491 ml/min (6 patients) and 432 ml/min (6 patients) The material is distributed according to age in Fig 9 It is clear from the diagram that the clearance of all 3 substances varied with the patients' ages

Quotient Hypaque/inulin clearance

The quotient Hypaque/inulin clearance was on the average 1 17 (45 periods) in the control series (A 1) with an observed range of 0 63—1 86 The corresponding mean quotient in the material for the patients studied with Probenecid (18 periods before Probenecid injection) was 1 19 with an

observed range of 0.89—1.40 The mean in series B was 1.25 (65 periods) with an observed range of 0.64—2.26 and for the total material a mean value of 1.21 (128 periods) In the statistical test (Student's t test) the Hypaque/inulin quotient differed significantly from 1.0 ($P < 0.05$) in all three series

Filtration fraction (Inulin/PAH clearance × 100)

The mean filtration fraction was 20 (39 periods) in the control series (A 1) and 23 (41 periods) in series B with observed ranges of 14—28 and 10—50 respectively

Quotient Hypaque/PAH clearance

The quotient Hypaque/PAH clearance in the control series (A 1) was 0.24 (48 periods) with an observed range of 0.13—0.47 and in series B it was 0.31 (41 periods) with an observed range of 0.13—0.81

Comparison between [131]*I Hypaque and inulin clearance*

In Fig 10 a the clearance of Hypaque in series A 1 is plotted against that of inulin It is clear that the majority of values were above the identity line except for the periods with high inulin clearance when the values were

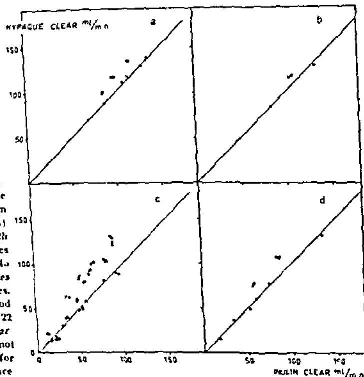


FIGURE 10 Relationship between Hypaque and inulin clearance in 15 controls (Series A 1) and 21 cases with renal disease (Series B): a Series A 1 45 period values b Series A 1 15 mean values. c Series B 65 period values. d Series B 22 mean values. The clearance values are not corrected to 1.73 m² body surface

below the 45° line The scatter diagram thus illustrates the above mentioned finding that the mean quotient Hypaque/inulin clearance exceeded 1 0 in the series In the diagram 35 of 45 values are on or above the 45°-line

Fig 10 c shows the same tendency in series B with 49 of 65 values on or above the 45° line

Figs 10 b and d give the mean values (of three periods) for the two series These scatter diagrams show the same tendency as those for the individual periods, but a much narrower scatter of the values

Comparison between quotient Hypaque/inulin clearance and inulin clearance

Figs 11 a—d show the quotients and mean quotients Hypaque/inulin clearance plotted against inulin clearance

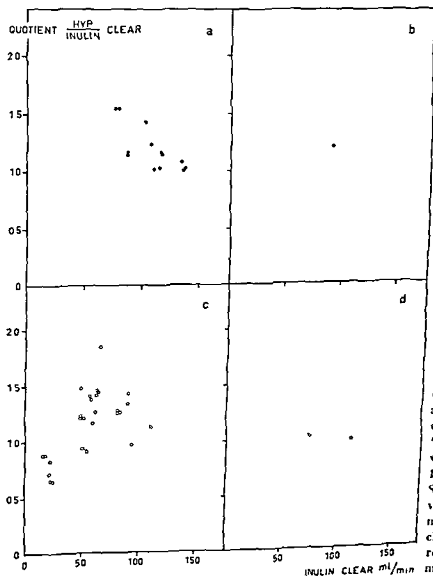


FIGURE 11 Relationship between quotient Hypaque clearance and inulin clearance in controls (Series A 1) and cases with renal disease (Series B) a Series A 1 45 period values b Series A 1 15 mean values c Series B 65 period values d Series B 22 mean values Inulin clearance is not corrected to hold for 1 73 m² body surface

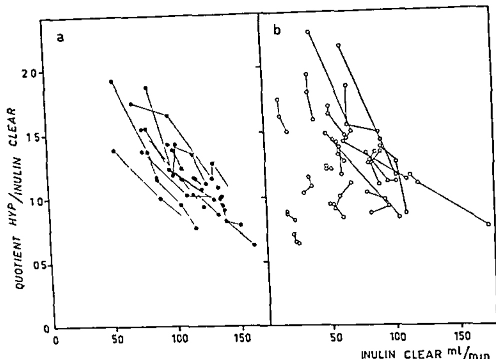


FIGURE 12 Relationship between quotient Hypaque inulin clearance and inulin clearance in 15 controls (Series A 1) and 22 cases with renal disease (Series B) Period values in individual cases joined by lines a Series A 1 b Series B

(period values and mean values for three periods) in series A1 and B

In Fig 11 a the negative correlation between the Hypaque/inulin clearance quotient and the inulin clearance suggests that the larger the inulin clearance the smaller the quotient The quotient decreases to 1 0 and sometimes even to a lower value when the inulin values are high In series B this relationship is not so distinct because of the wider scatter of the values This scatter in the pathological series may be due to the quotient values being artificially large at low clearance and therefore less suitable for analysis of this type

Fig 11 b shows the same linear relationship for the mean values as for the individual periods while Fig 11 d like Fig 11 c shows a less distinct tendency of the mean values in the pathological series

Figs 12 a and b give the quotient Hypaque/inulin clearance plotted against the inulin clearance where the *period values for the respective* patients are connected by lines to enable evaluation of the individual values in every case The relationship found for the entire series holds also for the individual cases The increase in the inulin clearance usually causes a decrease in the quotient

below the 45° line The scatter diagram thus illustrates the above mentioned finding that the mean quotient Hypaque/inulin clearance exceeded 1 0 in the series In the diagram 35 of 45 values are on or above the 45°-line

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Comparison between quotient Hypaque/inulin clearance and inulin clearance

Figs 11 a—d show the quotients and mean quotients Hypaque/inulin clearance plotted against inulin clearance

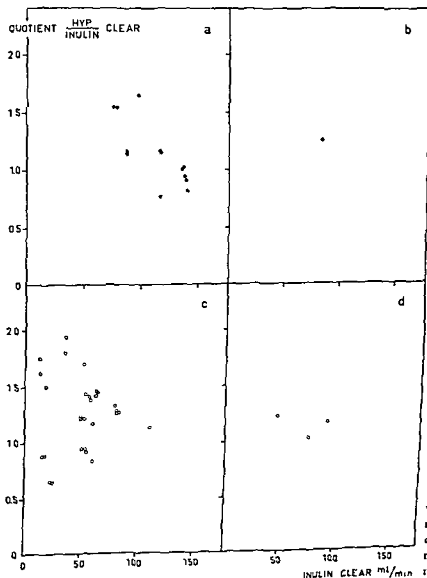


FIGURE 11 Relationship between quotient Hypaque clearance and inulin clearance in controls (Series A 1) and cases with renal disease (Series B) a Series A 1 45 period values b Series A 1 15 mean values c Series B 65 period values d Series B 22 mean values Inulin clearance is not corrected to hold for 1.73 m² body surface

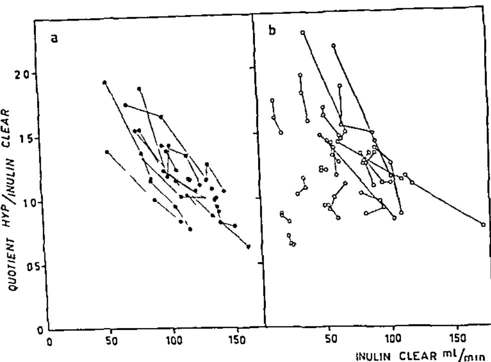


FIGURE 12 Relationship between quotient Hypaque inulin clearance and inulin clearance in 16 controls (Series A 1) and 22 cases with renal disease (Series B) Period values in individual cases joined by lines. a Series A 1 b Series B

(period values and mean values for three periods) in series A1 and B

In Fig 11 a the negative correlation between the Hypaque/inulin clearance quotient and the inulin clearance suggests that the larger the inulin clearance the smaller the quotient The quotient decreases to 1 0 and sometimes even to a lower value when the inulin values are high In series B this relationship is not so distinct because of the wider scatter of the values This scatter in the pathological series may be due to the quotient values being artificially large at low clearance and therefore less suitable for analysis of this type

Fig 11 b shows the same linear relationship for the mean values as for the individual periods, while Fig 11 d like Fig 11 c shows a less distinct tendency of the mean values in the pathological series

Figs 12 a and b give the quotient Hypaque/inulin clearance plotted against the inulin clearance where the period values for the respective patients are connected by lines to enable evaluation of the individual values in every case The relationship found for the entire series holds also for the individual cases The increase in the inulin clearance usually causes a decrease in the quotient

Studies with Probenecid (Series C)

Table XIV gives the initials, mg Probenecid given, number and duration of clearance periods, urine flow, Hypaque and inulin clearances and quotients Hypaque/inulin clearance

In this series interest was focused mainly on the changes in the quotient Hypaque/inulin clearance following the administration of Probenecid As is apparent from the table, cases B N V W and E C differed from the remainder in that they showed no depression of the quotient Hypaque/inulin clearance after administration of Probenecid As described on page 37, B N was the only patient who had discomfort during the examination The other two cases had rheumatoid arthritis, which had been

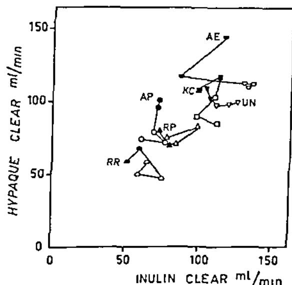


FIGURE 13 Hypaque clearance plotted against inulin clearance in study of Probenecid in 6 cases (Series C) Period values in individual cases joined by lines Solid symbols indicate clearance values before and open symbols after injection of Probenecid

treated with salicylic drugs These three cases were not included in Figs 13 and 14

The remaining 6 cases showed different degrees of depression of the quotient Hypaque/inulin after administration of Probenecid It should be observed that in R P and U N the *concentration of inulin was not quite* steady during the first two periods, so that the quotient Hypaque/inulin clearance was not quite constant In the remaining cases the concentration of both inulin and Hypaque were satisfactory during the examination

During the first two periods (before the injection of Probenecid) the Hypaque clearance was higher than the inulin clearance with the exception of the 2nd period in cases R P and U N During the last 3 periods (after injection of Probenecid) all inulin values were higher than the corresponding Hypaque values with the exception of case A N where the inulin values in the 3rd and 5th period were lower than the Hypaque values *The time of* the maximum depression effect on the quotient Hypaque/inulin varied somewhat, but in most cases it fell in the *4th period*

In view of the relation between the quotient Hypaque/inulin clearance and inulin clearance changes in filtration (inulin clearance) during the experiment must be taken into account in the investigation of the effect of Probenecid

In Fig 13 the Hypaque clearance in the 6 patients is plotted against inulin clearance Their period clearance values are connected by lines

which enable individual evaluation of the clearance series

Fig 14 gives the quotient Hypaque/inulin clearance plotted against the inulin clearance and here too the period clearance values are connected by lines It is clear from the two figures and Table XIV that in some of the cases the inulin clearance increased after the injection of Probenecid which *per se* can result in a lowering of the Hypaque/inulin clearance quotient In two of the cases (A E and U N) the decrease of the quotient was not larger than what might be explained by this increase in inulin clearance In the other 4 cases where the inulin clearance was largely unchanged the quotient decreased even to values below 1 0 Case B N was difficult to judge because of the side effects of the drug

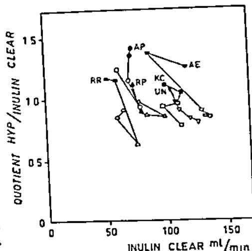


FIGURE 14 Quotient Hypaque/inulin clearance plotted against inulin clearance in study of Probenecid in 6 cases (Series C) Period values in individual cases joined by lines. Solid symbols indicate clearance values before and open symbols after injection of Probenecid

In patients V W and L C who received salicylica the inulin clearance was possibly decreased after the injection of Probenecid The quotient was not significantly changed in these tests but was if anything somewhat increased in the 4th period while the others showed maximal depression

E *Discussion*

The amount of radioactive Hypaque in clearance tests was calculated per kg bodyweight (0 6—1 2 μCi/kg) The total radioactivity during the test varied between 30—50 μCi These doses gave sufficiently high counting rates to ensure accuracy in the determination of the radioactivity The radioactive dose delivered to the gonads was so small as to imply no risk to the patients according to experimental experience and data obtained with ^{131}I labelled Urografin reported by Feine & Bauer (1961) A dose of 0 2 μCi/kg bodyweight for 24 hours implied a whole body dose of 1 8 mrad which they considered had been reduced to one third by rapid elimination of the substance during the first few hours 14 μCi for 24 hours implied a gonadal dose of 9—16 mrad in experimental phantom experiments and Feine & Bauer (1961) concluded that these doses found were much lower than those in excretion urography for example

The clearance of radioactive Hypaque at various plasma levels was of

the same order, but the *series* were too small to allow any statistical analysis. It was therefore not possible to draw any definite conclusions regarding differences between the series. Other investigators who used tracer doses with extremely low serum levels of Hypaque, also reported clearance values of the same order. Bianchi & Zampiere (1961) gave 0.6—0.9 μCi/kg bodyweight to 13 normal subjects with the intravenous infusion technique and obtained values of 86—188 ml/min with a mean value of 116 *ml/min*. Denneberg et al. (1961 a), who gave radioactive Hypaque with a small addition of non-radioactive Hypaque, found that in contrast to radioactive Diodrast, the clearance of Hypaque did not change at the serum levels used. Morris et al. (1965) found no difference in clearance between tracer doses of [131]I Renografin alone and combined with 60 ml of 60 per cent non-radioactive Renografin.

In clearance studies with [131]I labelled contrast media the purity of the radioactive solution must be considered. The occurrence of free radioactive iodide with its clearance of about 31 *ml/min might* be expected to give lower clearance values, as shown by Burbank et al. (1961) in a comparison between [131]I labelled Hippuran and PAH. No comparisons have been made between the clearance of radioactive and non-radioactive Hypaque. In the present investigation the radioactive preparations used contained more than 95 per cent radioactive Hypaque. These should give somewhat lower clearance values than non-radioactive Hypaque. For clinical use contamination of this order can be ignored as long as the percentage of free radioactive iodide in the preparation is known.

The mean inulin clearance in the control series was lower than in that described by Goldring et al. (1940). This was probably because of the considerable overrepresentation of elderly patients in the present series (50 per cent above 50 years). Davies & Shock (1950) reported a decrease in the clearance rate of inulin and Diodrast clearance with age. The inulin clearance found in the present cases fell within the observed and statistical limits (M + 2 SD) found in their material. The results obtained with [131]I Hypaque and PAH also showed a similar decrease of renal function with age, as judged from the mean values in the three age groups.

According to Lasser et al. (1962) the protein binding of Hypaque in man is negligible. This means that most of the Hypaque can be regarded as freely filtrable in the glomerulus. The question whether Hypaque is eliminated not only by filtration but also by tubular secretion was elucidated by comparison between Hypaque and inulin clearance. This comparison showed that although the values crowded around the 45° line and were closely related the majority of the Hypaque values were above the identity line in the scatter diagram. This means that the mean quotient Hypaque/inulin clearance in the material as a whole was larger than 1.0.

According to Smith (1951) it may

be accepted that the substance studied is excreted by the tubules in addition to being filtered through the glomeruli if the clearance of any substance studied which is not synthetized by the kidneys, is greater than the simultaneous inulin clearance in man i e when the substance studied/inulin clearance is greater than 1 0

Studies with the tubular blocking substance Probenecid showed that in those cases where the inulin clearance was largely unchanged a clear decrease occurred in the quotient This must be explained as a direct effect of Probenecid on the secretion of Hypaque

Why then does the quotient Hypaque/inulin vary inversely with the glomerular filtration rate (inulin clearance)? Assuming that Hypaque is freely filtrable and tubular secretion is fairly constant at different filtration rates the quotient will be higher at low *filtration* while a *higher filtration* will give a lower quotient but still above 1 0 If this quotient falls below 1 0 filtration alone plus secretion cannot explain this relationship but re absorption must be assumed Such a quotient below 1 0 was found *at high inulin values (see Fig 11)* and after blocking with Probenecid which not only lowered the quotient to 1 0 but also below this value

What is the interaction between these 3 mechanisms and what is the re absorption at different filtration *rates? It is possible that at low filtra*tion re absorption is also low while at high filtration re absorption is high which would thus mean that re absorption is constantly proportional to the filtration Such a *mechanism* would be in accord with Bojesen s (1954 b) theory of the salt and water *re absorption in* the tubules of the mammalian kidney Bojesen (1954 b) showed that as far as the excretion of water is concerned it is possible that tubular re absorption *capacity* determines *the order of the filtration* and that there is a proportional relation between the *filtration and the re absorption* This explains why the quotient Hypaque/inulin at low filtration exceeds *1 0* (*when tubular secretion* is greater than re absorption) and at high glomerular filtration rate below 1 0 (when tubular secretion is less than re absorption)

These *observations* thus suggest tubular re absorption of Hypaque This seems to explain the delay of the initial excretion of Hypaque compared with inulin which has been reported *in studies using the single injection* technique (Denneberg et al 1961 a)

It should however, be pointed out that this possible re absorption could not be determined quantitatively from available data *It is reasonable to* assume that estimations based on the depression of the Hypaque/inulin quotient below 1 0 *in the Probenecid* studies must lead to *corresponding* low values There are two *reasons for* this it was not possible to use maxi*mum doses of Probenecid because of* side effects and it is not certain that Probenecid can block tubular secretion completely

The renal excretion *mechanism* of Hypaque (*Urografin or Renografin*) has been studied in animal experi

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ments with different methods (Chapter II) These investigations have given divergent results It was found that *glomerular filtration* dominated, while the question of possible tubular secretion and also the possibility of reabsorption was left unanswered In 13 normal subjects Bianchi & Zampiere (1961) found that the quotient between radioactive Hypaque and thiosulphate clearance was 0 91 ± 0 15 with an observed range of 0 74—1 21 Bianchi & Toni (1963) reported a comparison between radioactive Hypaque and inulin in subjects with and without renal diseases They found a good correlation between the clearance of the two substances, but they gave no details In their scatter diagram 16 of *the 35 observed values were above the* identity line (*i e* quotient Hypaque/inulin was above 1 0) 4 along the line and 15 below the line

Tauxe et al (1964) reported similar results with inulin and radioactive Hypaque clearance, with only a narrow scatter around the 45° line They examined 47 patients with suspected renovascular hypertension and found a mean difference of 2 2 ml/min per 1 73 m^2 between these substances with a correlation coefficient of 0 99 and a mean Hypaque/inulin quotient of 0 96

Recently Morris et al (1965) compared ^{131}I labelled Renografin and inulin on *13 subjects with and without* renal disease They found a mean Renografin/inulin quotient of 1 04 and an observed range of 0 76—1 86 (69 clearance periods)

No explanation can be offered for the difference between the results in the abovementioned investigations and the present study Only at high glomerular filtration rate was agreement found with the previous investigations Of the factors responsible for too low a Hypaque/inulin clearance quotient in the investigations referred to above, it is possible that one was a higher degree of impurity of the radioactive preparation and the other a competition effect of some other substance (*e g* PAH) in the infusion solution Only Morris et al (1965) have given data on the purity of the preparation (2 per cent free radioactive iodide in the solution), while Tauxe et al (1964) reported that despite various techniques, their results were inconclusive when labelled diatrizoate was assayed *for purity This might indicate that* they used a preparation with a high percentage of free iodide and other impurities In a previous investigation on dogs they had found 5 per cent iodide plus 5 per cent non identified impurities and they reported this as a cause of the difference found between Hypaque and creatinine clearance (Blaufox et al 1963 d)

It should be pointed out that the comparison between Hypaque and inulin clearance shows the net result of all processes involved in clearance mechanism and not the individual processes acting in different directions *(tubular secretion and reabsorption)* The fact that a substance has a clearance identical or nearly identical with that of inulin is no proof that it is excreted by glomerular filtration only This has been shown by Lindahl & Josephson (1945) with the substance

sulpha methyl thiodiazole. The essential thing is not whether the quotient Hypaque/inulin clearance is 1 0 but whether it is depressed and falls after administration of a tubular blocking substance When it does it is a sign of tubular secretion of the substance

Radioactive Hypaque and Renografin have been tried experimentally as a suitable substance for the single injection clearance (Bianchi 1961 Dennebеrg et al 1961 a Blaufox et al 1963 d Meschan et al 1963 a) and as a substitute for inulin in the measurement of glomerular filtration by the intravenous infusion technique (Tauxe et al 1964 Morris et al 1965) The results obtained in the present investigation show that Hypaque is eliminated mainly by glomerular filtration while the tubular secretion and reabsorption more or less compensate one another This means that even if Hypaque does not give a true measure of glomerular filtration it should be clinically useful as a substitute for inulin until more suitable substances have become available Hypaque is also a suitable substance for the single injection clearance because the clearance appears to be independent of the plasma level This indicates that the Hypaque clearance by the single injection technique is possible despite simultaneous tubular secretion and reabsorption

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CHAPTER VIII

THE RADIOISOTOPE HYPAQUE RENOGRAM QUANTITATIVE EVALUATION AND RESULTS IN RENAL DISORDERS

Introduction

As pointed out in Chapter II E, the choice of the most suitable ^{131}I-labelled substance for renography offered considerable problems. The advantages and disadvantages of the various substances made themselves felt in both experimental and clinical comparisons. Promising clinical results with the three radioactive diatrizoate products (Hypaque, Renografin, Urografin), were reported by Denneberg & Hedenskog (1959 a), Winter et al. (1959), Denneberg (1959/60), Bauer & Feine (1960), Abt & Balkus (1961), Gasser & Hawliczek (1962), Montandon et al. (1962), Gasser et al. (1963), Koecke & Bauer (1963), Staehler et al. (1963) and others.

A considerable problem common to methods using ^{131}I labelled substances for renography is the difficulty in interpreting and obtaining quantitative measures of renal function from the curves.

The purpose of the investigation described in this chapter was to elucidate the clinical application of the renogram in a series of patients with and without renal disease. On the basis of a theoretical analysis of the complex external renal curves, simple parameters were selected to allow quantitative evaluation of the renogram and definition of criteria of normal and abnormal curves.

A. Material

The material is summarized in Table VI with respect to disease, number of patients, sex, age (range and means) and number of renograms and roentgenographic examinations (see also Table XX).

Series 1. Controls

This series consisted of ten healthy students and 21 hospital patients with diseases unrelated to the kidney and cardiovascular system and being cared for at the medical and surgical departments, Malmö general hospital. None of the patients in this series had clinical or laboratory evidence of renal disease. They were normotensive and afebrile.

Table VI Survey of material used in renogram studies

Series	No of cases			Age (years)		No of	
	Total	Female	Male	Range	Mean	Reno grams	Roentg exam
A Control	31	3	28	17—57	33 2	41	
B Chronic glom nephritis	8	5	3	30—49	39 1	8	2
C Chronic pyelonephritis	18	15	3	19—65	48 2	18	13
D Polycystic disease	4	1	3	21—44	35 0	4	4
E Other parenchymal diseases	18	6	12	19—60	41 1	18	14
F Hypertension	10	6	4	30—67	47 1	10	10
G Nephrectomy or aplasia	6	4	2	17—64	47 5	6	2
H Acute ureteral obstruction	33	13	20	14—72	42 0	33	32
I Hydronephrosis	13	5	8	33—69	52 0	31	13
Total	141	58	83	14—72	41 9	151	90

Series B Chronic glomerulonephritis

The eight patients in this series had been admitted on several occasions to the medical department for investigation and treatment of chronic glomerulonephritis which in six of the eight cases started after angina tonsillaris with acute glomerulonephritis In two of the cases a transient nephrotic syndrome had preceded the chronic stage Post mortem examination was performed in *seven* of these *eight* cases and in all of them the clinical diagnosis was confirmed

Series C Chronic pyelonephritis

The 18 patients in this series had a history of several attacks of acute pyelonephritis and later chronic pyelonephritis In five cases (V L A T A D O A M W) there was a history of roentgenographically verified urinary obstruction and renal stone, while the others had no history of renal stone Some cases also had roentgenographic signs of chronic pyelonephritis satisfying the criteria given by Dejdar (1959) The majority of cases had impairment of renal function and clinical signs of chronic pyelonephritis at the time of renography In nine of the 18 cases the clinical diagnosis was verified at autopsy

Series D Polycystic renal disease

Three of the patients in the series had polycystic renal disease in their family history and the clinical diagnosis was verified at autopsy In the fourth case (K P) there was no family history but the diagnosis was verified at surgical exploration

Series E Other parenchymal diseases

This series of 18 patients was *more* heterogeneous in respect to the causes of the diseases than the preceding series The patients had been admitted *to the* medical department for investigation of parenchymal renal disease with varying pathological findings (proteinuria haematuria, bacteriuria

impairment of renal function of obscure origin) and were cared for under the diagnosis of chronic nephritis or nephropathy of obscure origin In several of the cases the diagnosis was suspect quiescent pyelonephritis, but since these cases did not fill the criteria of pyelonephritis they were assigned to this series In three of the cases the patients had coexisting diabetes mellitus Autopsy or laparotomy was performed in three of the 18 cases

Series F Hypertension

This series consisted of ten patients with manifest hypertension, without previously known renal parenchymal disease All the patients had been admitted on at least one occasion for extensive investigation and care but no endocrine disease or coarctation of the aorta could be demonstrated A diagnosis of hypertension required demonstration of a diastolic blood pressure above 95 mm Hg and a systolic blood pressure above 150 mm Hg at rest (the values are based on the diurnal blood pressure recordings during spells in hospital) and ocular fundi of hypertensive type of at least grade II according to Keith Wagener & Barker (1939) In two of the cases (Ö N and J G) the clinical diagnosis was verified at autopsy where bilateral nephrosclerotic changes were found J G and Å N were the only patients who had a malignant hypertension with a short history and rapid progression, while the remaining eight patients showed the clinical picture of benign hypertension

Series G Aplasia or post nephrectomy state

Two of the six patients in this series had at previous examination been found to have only one kidney, while the remaining four had undergone nephrectomy for various indications (hydronephrosis, unilateral silent kidney, pyelonephritis) In one of the cases with renal aplasia the clinical diagnosis was verified post mortem

Series H Acute ureteric obstruction (suspect or certain)

The series comprised 33 patients referred to the surgical department because of suspect acute renal obstruction After clinical examination they were referred for renography, which was done immediately, and then for excretion urography Nine patients had been given spasmolytics before or on admission to the surgical department because of severe acute pain, and 19 had pain at the time of the examination (renography or urography)

Series I Hydronephrosis

This series consisted of 13 patients with hydronephrosis of varying origin The series was much more heterogeneous in respect of origin than that of acute ureteric obstruction Some of the patients were examined because of suspect renal stone with previous characteristic pain in their histories some had previously known renal stone and had become worse and were to be examined and if considered necessary offered operation some had a silent kidney and prostatic cancer

and some had gynaecological cancer which was operated upon and treated with radiation. Common to the entire series was the fact that all of them had unilateral or bilateral roentgenological changes suggestive of hydronephrosis.

Of the cases with renal disease 86 were cared for at the medical department, 41 at the surgical department (including 33 ambulatory), two at the department of gynaecology, two at the department for infectious diseases (see appendix).

Some of the cases accounted for here have been presented in previous publications (Denneberg & Hedenskog 1959 b, Denneberg 1959/60), namely L. A., Ö. P., V. P., H. H. and the cases in the series of acute ureteric obstruction except G. B., T. A. and G. R.

B. Technique

Test substance

^{131}I labelled Hypaque was used in the renogram studies. The composition and dilution of the radioactive solution (solution A) used in all cases except those in series H, which were examined with solution B, are given in Chapter IV.

The radioactive dose injected was 0.4 μCi/kg bodyweight. Preparation of standards and handling of the stock solutions is described in Chapter V.

Equipment for external measurement of radioactivity

Two scintillation detectors with a 1" by 1" thallium activated sodium iodide crystal were used for measurements over the kidneys. The scintillation crystals were placed in a cylindric lead shield with a conic collimator with an inner diameter of 50 mm and an outer diameter of 65 mm. The distance between the crystal and the opening of the collimator was 35 mm and the angle of the collimator insert was 110° (Fig. 16). The two detectors were mounted on a stand which allowed adjustment in the horizontal and the vertical planes. The arrangement is illustrated in Fig. 15.

A third scintillation detector in a cylindric lead collimator with a channel length of 110 mm and collimator opening 60 mm in diameter was used for external measurements over the heart. The detectors were connected to ratemeters which were coupled to a 6 point recorder with fixed paper speed of 75 cm per hour. The 6 point recorder had a speed of 5 dots/minute and curve (chest curve or one of the two renal curves). The time constants could be set at 0.5, 2.5, 10 and 40 seconds.

Preparation of the patients and roentgenographic localisation of the kidneys

The patient was examined at the ~~roentgen diagnostic~~ department in the morning on the examination day for marking of the site of the kidney. The patient was instructed to drink before the examination and the only exceptions to this rule were the patients with acute urinary obstruction who were

impairment of renal function of obscure origin) and were cared for under the diagnosis of chronic nephritis or nephropathy of obscure origin. In several of the cases the diagnosis was suspect quiescent pyelonephritis, but since these cases did not fill the criteria of pyelonephritis they were assigned to this series. In three of the cases the patients had coexisting diabetes mellitus. Autopsy or laparotomy was performed in three of the 18 cases.

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Series G. Aplasia or post nephrectomy state

Two of the six patients in this series had at previous examination been found to have only one kidney, while the remaining four had undergone nephrectomy for various indications (hydronephrosis, unilateral silent kidney, pyelonephritis). In one of the cases with renal aplasia the clinical diagnosis was verified post mortem.

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The series comprised 33 patients referred to the surgical department because of suspect acute renal obstruction. After clinical examination they were referred for renography, which was done immediately, and then for excretion urography. Nine patients had been given spasmolytics before or on admission to the surgical department because of severe acute pain and 19 had pain at the time of the examination (renography or urography).

Series I. Hydronephrosis

This series consisted of 13 patients with hydronephrosis of varying origin. The series was much more heterogeneous in respect of origin than that of acute ureteric obstruction. Some of the patients were examined because of suspect renal stone with previous characteristic pain in their histories, some had previously known renal stone and had become worse and were to be examined and if considered necessary offered operation, some had a silent kidney and prostatic cancer

curve had become more stable (about 30 minutes after injection) a time constant of 40 seconds

Comments

The renal detectors were calibrated daily and adjusted to register the same level of radioactivity with a standard source of $Na^{131}I$ When a test dose of 0.4 µCi ^{131}I Hypaque/kg bodyweight was used a peak count rate of 10,000 cpm was obtained in normal subjects The variation of the graphic recorder was found to be less than 2 per cent from the mean value

To check that the detectors were placed over the kidneys during the measurements *i e* where the count was highest control measurements were made at *the end of the examination* by manual scanning of the back This control gave good agreement with the marking of the kidneys and only in eight examinations were new renograms necessary on the following day with the use of positions found by scanning (cases I B H H M I S B H Z A A Z N)

Exceptions to these principles with indication and X ray film were made in the series with suspect acute ureteral obstruction where the radioisotope test was performed before urographic examination in the acute stage of disease When the *patients in this series* were acutely ill with severe pain and in a poor general condition the measurements over the kidney were made for only 15—20 minutes. On examination of this series manual scanning was done after the initial phase of the curve (about 20—30 seconds after injection) Immediately after the highest count was found, the definitive measurement was continued Also in this series the detectors were checked *regarding their positions* over the kidneys by control scanning after the renogram test and here too good agreement was also found with but few exceptions (cases A B C N E R N W) in which scanning was repeated during the test because the patients had moved during pain

Control of detector equipment

The shielding effect of the renal collimator was examined in two types of model experiments In the first experiment 10 µCi ^{131}I labelled Hypaque in a *tube with a volume of 1.0 ml* was used as a source of radiation The tube was moved in the vertical and horizontal planes in a tank filled with water during registration of the radioactivity In this examination the front of the collimator was 1 cm from the surface of the water (Fig 16)

In the second experiment a phantom of the torso urinary tract in water was built up simulating the conditions prevailing at renography The torso was represented by a plastic 10 litre tank The kidneys were represented by two 250 ml flasks (B and C) each containing 150 ml of water They were submerged in the torso with their mid points 8 cm from the posterior surface of the phantom and 12 cm apart. A third flask (D) with a volume of 250 ml was placed in the midline 12 cm below and 10 cm anterior to the

examined without any previous examination at the roentgen department.

The approximate position of the kidneys was marked with indicators on the patient's back, after which a roentgenogram was taken of the kidneys with the patient sitting upright. With the aid of the position of the indicators relative to the outlines of the kidney on the roentgen film it was possible to outline the kidneys on the patient's back. When the roentgen film failed to outline the kidneys the examination was repeated with longer exposure until the outline was satisfactory. In all patients examined urographically before renography the position of the kidneys was estimated in order to check that the patient had not a pelvic kidney, and to compare the sizes of the two kidneys in excretion urograms and in films with the patient sitting upright.

The examination procedure of renography

The tests were carried out in the morning between 8.00 and 9.00 a.m. with the patient sitting comfortably during the test, leaning slightly forward with a shielded scintillation detector placed against the chest. The detector was held in the front in the midline with the collimator close to the skin and the cranial edge of the outer opening at the manubrium sterni.

The two detectors over the kidneys were placed with their apertures in contact with the back of the patient. The detector units were directed obliquely downwards (about 45°) and outwards (about 15°) from the mid line (see Fig. 15).

Injection. The dose of radioactive substance was injected intravenously into a cubital vein and, to avoid extravascular injection of the radioactive substance and at the same time standardize the injection technique, blood was always aspirated in such an amount in the syringe (1 ml Mantoux syringe) that the latter was filled before the injection, which was then given during a period of 5—10 seconds. The injection was never accompanied or followed by any complications.

Recording. Recording of the chest and renal curves was continued for 45—60 minutes. During the first 10—15 minutes a time constant of 2.5 seconds was used and then a time constant of 10 seconds and after the

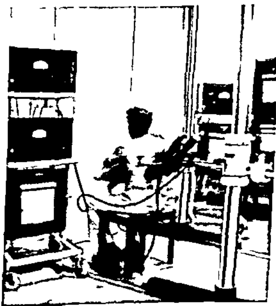


FIGURE 15. Arrangement for determination of the ^{131}I Hypaque renogram.

Results and comments

The measurements showed that each detector measures also 5 per cent of the activity in the contralateral kidney and 6 per cent of the activity in the bladder. This means that every renogram is influenced to some extent by the activity in the contralateral kidney. In practice, however, this influence is negligible, as is clear from the following example where the influence was maximal. In case L. A. (right renal aplasia) the counts for the calculation of uptake ratio on the right side were 4800 and 3900 cpm on the right side and 6400 and 8000 cpm on the left. The uptake ratio on the right side was 0.81. If the values are corrected for the 5 per cent of the simultaneous counts from the left side, the ratio will be 0.78 instead, *i. e.* an error of only 4 per cent. In all of the cases with a smaller difference between the two sides the error must therefore be still smaller.

During the first minutes after the injection the bladder activity was low and could therefore hardly influence the uptake ratio. It must, however, later disturb the *excretion ratio* but is least when excretion is impaired. The bladder activity and the body background probably help to explain why the excretion segment of the renogram falls slower towards the end of the test.

Roentgen examinations

The roentgen examinations were done at the roentgen diagnostic department by routine methods (determination of the size of kidney, excretion urography, retrograde pyelography, selective renal angiography or aortography).

In the examination of the cases with acute ureteric obstruction, where urography was done in the acute stage of the disease, the patients were examined without preparation and compression. Neither was any compression applied in this examination of patients with hydronephrosis, while compression was used in patients with parenchymatous renal disease. For the roentgen examinations 20 ml of 45 or 60 per cent Urografin (Schering) was used.

Other laboratory studies

Determination of serum creatinine (normal values 0.5—1.2 mg/100 ml) was made according to the method described by Haugen & Blegen (1952) and modified by Løken (1954).

In the determination of endogenous creatinine clearance the urine samples were collected during a 4 hour period and determinations of the endogenous plasma creatinine were made. Only voided urine was used. Normal values 90—148 ml/min. The creatinine clearance is corrected to hold for 1.73 m² body surface area.

Determination of the serum N. P. N. was done according to the method of Rappaport & Eichhorn (1947). Normal values 20—40 mg/100 ml.

The test of maximal specific gravity was done according to the method described by Addis & Shevky (1922).

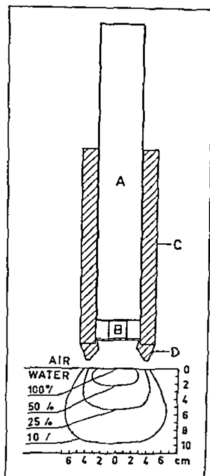


FIGURE 16 Scintillation counter used for renography and isoresponse curves in water A Scintillation detector B Crystal C Lead shield D Lead insert of collimating aperture

kidneys representing the urinary bladder (Fig 17) The radioactivity was measured with the two scintillation detectors whose collimators were in contact with the surface of the torso All counting rates were corrected for background The experiments were done both with and without radioactivity in the torso outside the flask In three experiments, 1 3 and 10 μCi radioactive Hypaque respectively was added to the water in the torso The right flask was at the same time given a series of increasing doses of radioactivity (1, 2, 3, 4, 6, 8, 10 μCi) while the left flask was not given any radioactive solution during the entire experiment The activity from the detector over the left flask was expressed in per cent of the activity from the right

In the experiment with the "bladder" neither the torso nor the "kidneys" contained any radioactive solution The radioactivity was determined while the bladder was receiving a series of increasing amounts of radioactive Hypaque (1, 2, 4, 6, 8, 10 μCi) The bladder was empty from the beginning of the experiment but was given 5 ml water together with every dose, and at the end of the examination it contained 30 ml

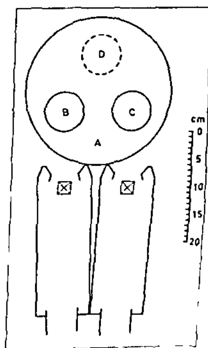


FIGURE 17 Phantom torso urinary tract used for study of shielding effect of the renal collimator A Plastic tank B and C Flasks representing the two kidneys D Flask representing the bladder

ney On experimental ligation of the ureter the tracing showed a continued rise

It is generally accepted that the second segment is an approximate measure of renal function and the third segment is related mainly to renal drainage The opinion put forward by Taplin et al (1956) and Winter (1959) that the initial segment is a measure of vascular capacity has however been assailed by other investigators

Wax & McDonald (1962) who used ^{131}I Hippuran examined the vascular capacity of the kidney and the background from the surrounding tissues in dogs and humans The background was determined with radioactive albumin and measurements were made before and after nephrectomy They found that at 1 minute i e close to the time of the spike of the vascular segment only 16 per cent was contributed by vascular capacity and 59 per cent by the functional components At the time for the maximum height of the curve the vascular capacity was only 5—10 per cent of total height at that time The transfer system of ^{131}I Hippuran could be competitively inhibited by administration of sufficient doses of PAH The effect caused a marked decrease in amplitude of both the initial rise and maximum height of the curve and also a slowing of the excretory segment by flattening of the curve This led to the conclusion that a large fraction of the initial segment represented tubular uptake and that less than half of it reflected inflow of isotope containing blood Klapproth et al (1962) performed similar experimenst on dogs and obtained the same results

Wax & McDonald (1962) also showed that after administration of Probenecid the second segment of the curve was depressed as much as 70 per cent There was an excellent correlation with the magnitude of this depression and the reduction of the clearance of PAH Similar results by saturation with PAH have been reported by Montandon et al (1962) in rabbits and zum Winkel (1964) in rats with radioactive Hippuran and Hypaque renography

Klapproth et al (1962) showed that osmotic diuresis increased urine flow sharply and depressed the amplitudes of the renogram On decrease of the flow of tubular fluid by ureteric occlusion or by occlusion of the renal vein the curve rose continuously Klapproth et al (1962) concluded that during the second segment of the *curve which* had been assumed to represent tubular accumulation of the isotope much of the isotope was accumulated not only in tubular cells but also in tubular fluids

A *number of investigations* have been carried out on various animals to study the obstruction of the urinary pathways and its effect on the renogram especially on the third *segment* (O Conor et al 1961 *Klapproth et al* 1962 *Kretchmar & Greene 1963*)

Taplin et al (1956) showed that ligation of the ureter resulted *in a con*tinuously rising curve as a sign of accumulation of the isotope *in the kid*ney without any *signs of excretion*

Lowest normal specific gravity 1 025
Urine volume < 400 ml

The values for endogenous creatinine clearance and serum creatinine or N P N and concentration tests were determined during the same hospital spell as the isotope test, but at an interval of 0—7 days between the examinations The corresponding interval between renography and roentgenography was 1—14 days

C Clinical evaluation of radioisotope renogram

Evaluation of the external renal curves has been the subject of much debate in recent years and several types of procedures have been described or suggested The discrepancies between the results of these analyses have made it difficult to decide which parameters are most useful for clinical use A brief summary of experimental data obtained in animals and in human beings therefore appears necessary for the evaluation and interpretation of the renogram segments and their changes under different experimental conditions

Experiments in animals

Our fundamental knowledge of the different appearances of the renograms made under different experimental conditions has been described in various publications (DeMaria et al 1960, Denneberg et al 1960 Whitley et al 1961, zum Winkel 1961 Abbassian 1962 Magnusson 1962 zum Winkel et al 1962, Beall et al 1963 Blaufox et al 1963 a, Wisenbaugh et al 1965)

zum Winkel (1964) gave a detailed description of a number of these experiments He also reported personal experiments with ^{131}I Hippuran which were in part a further link of previously published experiments with various radioactive contrast media (Diodrast, Hypaque, Hippuran)

Taplin et al (1956) discussed the different segments of the renogram on the basis of animal experiments They thought that the initial segment of the curve (vascular segment) represented the vascular capacity plus that of the background tissue When a detector was placed over the area where a kidney had been removed the spike of the initial segment was approximately one third of that obtained over a normal kidney The secondary rise in the tracing beginning during the second or third minute and lasting three to five minutes (uptake segment) was due to tubular cell secretion of the contrast medium Therefore the steepness and height of this segment was proportional to renal function This segment was taken as a good index of total function of the kidney When the kidney was absent or non functioning or when the renal artery was blocked a secondary ascent did not occur The second segment terminated at a maximum value after which the third segment (excretion segment) started 5—8 minutes after injection of the substance There was a rapid fall for about 5—10 minutes and this fall represented elimination of the radioactive urine from the region of the kid

accumulation of the radioactive substance in the kidney and intrarenal passage was shown in the experiments with ligation of the renal artery, inhibition by Probenecid or saturation with unlabelled PAH. These experiments (particularly in the [131]I Hippuran renogram) indicated that the second segment was closely correlated with renal blood flow (vascular capacity) as well as with the concentrating capacity of the kidney. This segment was affected also in its latter part by changes in renal drainage. The functional capacity of the kidney could thus be judged best from the second segment.

The second segment terminated at the maximum height of the curve. This proved to be dependent on variations in urine flow and changes in drainage, *e. g.* by ureteric obstruction or venous occlusion. The amplitude of this maximum value of the curve increased when urine flow was low and the drainage impaired but decreased when it was high and the tubuli blocked.

From experiments with ligation of the ureter and low urine flow it was clear that the third segment was an expression for the elimination of the isotope from the region of the kidney, *i. e.* a measure of evacuation from the field of vision of the detector. This segment elucidated mainly the disturbances in drainage. During this segment, however, uptake continued although elimination played a dominant role.

The results showed no essential difference between animals and human beings in the build up of the renogram. The experimental studies may thus serve as a basis for interpretation and evaluation of the different segments of human renograms.

Experimental studies have shown that renograms are extremely complex. No direct measures of the components of renal function such as glomerular filtration, tubular secretion or renal blood flow could be recognised from the curves. On the other hand it was shown that the second and third segments were correlated with the accumulation of the test substance in the kidney and elimination of it from the region of the kidney. No distinct limits could be determined regarding the time of the start or end of uptake or excretion. The curve seems to "reflect a kinetic equilibrium of several aspects of the movement of renal fluids and of the tubular transport not interpretable in terms of specific renal functions" (Klapproth et al. 1963).

Technique of evaluation of renograms by other investigators

Investigations in animals and in man have shown that the renogram is a very complex curve and does not allow evaluation of glomerular filtration or tubular secretion separately. Attempts have however been made to obtain quantitative measures from the renogram. *Changes in one or more of the* segments have been interpreted as signs of different types of disturbances of renal function. The purpose of the present analysis was to *find more* objective criteria of normal and ab

Magnusson (1960) showed that the curve had a normal uptake segment, which meant that the capacity of the kidney to take up the isotope was intact Denneberg et al (1961 b) showed that when the ureter was ligated for any length of time, pathological changes occurred and severely interfered with renal function This was reflected particularly by the decreased uptake of [131]I Hypaque and by the retarded excretion After eight weeks' ureteric ligation the kidney was probably not able to take up the test substance

Studies in human beings

Investigations in man have been carried out under various experimental conditions to study the renogram and its various segments In principle similar methods have been used as those in animal experiments, but modified for human beings (Winter & Taplin 1958 Meade & Shy 1961, Montandon et al 1962, DeMaria et al 1963 Hine et al 1963)

As in the animal experiments, much interest has been focused on the evaluation of the initial segment as a measure of vascular capacity Wax & McDonald (1962) carried out some of their investigations on patients under conditions described in the experimental part This view that the initial segment reflects only part of the vascular capacity together with the non renal vascular capacity and also tubular uptake was verified by Dore et al (1963) and Wedeen et al (1963)

Wax & McDonald (1964) demonstrated a good correlation between the maximum height of the curve and PAH clearance in their clinical study Dore et al (1963) found that different sized doses of PAH exceeding Tm PAH progressively flattened the second segment They compared the findings in this experiment with the results of measurements over the kidneys and the bladder (cystogram) They found that almost no tracer left the renal area during the second segment of the renogram Since the tracer was retained in the kidney during this segment and since low plasma concentrations of [131]I Hippuran are almost completely removed the rate of tracer accumulation or the slope of this segment of the [131]I-Hippuran renogram was an index of renal blood flow

Conclusion of renogram experiments

The results obtained in the experimental investigations and reported above can be summarised as follows measurements performed before and after nephrectomy, experiments with RISA and injection of large doses of unlabelled PAH, showed that about one third of the first segment represented body background The rest of this segment represented renal blood content and a considerable functional component which argued for accumulation in the kidney These data showed that the first segment was much more complex and did not represent the vascular capacity of the kidney to the extent originally supposed

That the second segment of the curve was mainly an expression of the

has been described by Taplin et al (1956) Winter (1956 1957 1959 1963) Tauxe et al (1962) Doig et al (1963) Pedersen et al (1964) and others

2 *Comparison with group envelopes*

With this method several normal curves are plotted and an envelope is drawn which includes all these curves The actual curve is then compared with this normal field (Meade & Shy 1961)

3 *Calculation of different types of ratios, slopes tangents, angles from one or the other of the three segments of the curve*

Common to these parameters is that they give a measure of the slope of selected parts of the curve Applied to the second or third segment the parameters can for example be used as measures of uptake or excretion of the substance An example of such an up take ratio is that between the two amplitudes of the curve $a_{2\ min}/a_{B}$ (zum Winkel 1964) A corresponding measure calculated from the excretion segment *e g* the amplitude at 10 min ($t_{10\ min}$) in relation to the amplitude at maximum count of the curve (a_{max}) was used by Wedeen et al (1963) A further variant is the amplitude 15 min after the maximum count (a_{I}) in relation to the amplitude at maximum count (a_{max}) The ratio is then multiplied by 100 Subtraction from 100 per cent gives the difference per cent of relative retention (Iarmelant et al 1964)

The slopes can be expressed as an angle or as a tangent, *i e*, a measure of the rate at which the substance is concentrated or excreted in the area studied These values have been determined and expressed as an angle or tangent of the original curve and one of its segments and partly by measurement *of the half time of the excretion* or uptake segment The latter determination has, as a rule been used after replotting the data on semilogarithmic paper with extrapolation of the slope of the third segment to its intercept on the ordinate. At different intervals the uptake value measured is subtracted from the excretion slope and the differences re plotted after which the half time value of the exponential line is determined (Block et al 1960 a Roth et al 1960 Witcofski et al 1961 Magnusson 1962 Becker et al 1963 Dawborn & Doyle 1963 Taplin et al 1963 Krogsgaard & Friis 1964)

4 *Determination of parameters from the time axis of the curve*

This method gives durations of the different segments Examples are the time to maximum count ($t_{max.}$) *i e* interval between injection and the time of maximum count or from the injection to 75 per cent of the maximum count (t_{E}) or 50 per cent of maximum value (t_{G}) etc. (Spencer et al 1961 Stewart & Haynie 1962 Brown et al 1963 Johnson & Odom 1964 Krogsgaard & Friis 1964)

A special method for analysis of the first segment has been described by

normal curves, preferably from parameters easy to calculate without complicated analysis of the curves

Taplin et al (1956) and Winter (1956, 1957, 1959) thought that renography should be regarded simply as a qualitative test allowing no quantitative analysis In the evaluation of the renogram they considered the general shape of the curve and regarded the first *segment as an indicator of vascular capacity But as men*tioned in the experimental investigations, the first segment proved a poor measure of vascular capacity because of the considerable extrarenal and functional components The initial *segment has proved less suitable for* the evaluation of vascular capacity in clinical practice, too (Stewart & Hay*nie 1962,* Dawborn & *Doyle 1963,* Farmelant et al 1964)

Most investigators have also preferred to calculate their parameters from the *second* or *third segment* of the curve Different types of parameters have been chosen and applied to subjects with and without renal disease and the results have been compared with the clinical findings renal function data and different types of roentgen examinations The examiners have selected one or usually a combination of several parameters to characterise their curves

The methods used are briefly outlined below and Fig 18 shows reference points used by various investigators in the analysis of the renogram Some investigators have defined the 0 point (t_0) from the beginning of the injection, others at the end of the injection, but also at the time when the initial segment of the curve rises above background activity In the figure *point 0 is set at the end of the injec*tion

1 *Visual comparison of the curves*

This method compares the curves regarding (a) the changes in the three segments, which occur in various renal *diseases and in different degrees of* impairment of renal function (b) the amplitudes of the curve at different times The amplitudes measured were the maximum value of the first segment (a_B), the height at maximum *count* (a_{max}) and the height at some selected time of the excretion segment, *e g* 10, 15, 20 or 30 minutes ($a_{10\ min}$ $a_{15\ min}$, $a_{20\ min}$, $a_{30\ min}$) Evaluation according to these principles

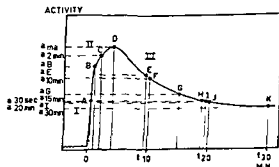


FIGURE 18 Reference points used by various investigators in the analysis of the renogram O Time of injection I 1st segment II 2nd segment. III 3rd segment Amplitudes A 30 seconds after injection B of 1st segment C 2 minutes after injection. D at maximum height. E 75 per cent of maximum height F 10 minutes after injection G 15 minutes after injection. H 50 per cent of maximum height I 15 minutes after maximum height J 20 minutes after injection. K 30 minutes after injection

procedure with amplitudes and used parameters of the types described under points 3 and 4 instead Brown et al (1963) and Krogsgaard & Friis (1964) used t_{max} respectively 50 per cent of maximum value (t_G) while a number of other examiners used t_{max} in combination with one or more other parameters to describe the renogram (Roth et al 1960 Meade & Sky 1961 Boyd & Murdock 1962 Wedeen et al 1963 Johnson & Odom 1964 Scholtz et al 1964 zum Winkel 1964 and others)

In a few large clinical series the investigators have compared their own evaluation technique with those used by others Stewart & Haynie (1962) reported that the height of the first segment (a_B) was poorly correlated with renal artery stenosis as well as the maximum height of the curve (a_{max}) and the value 15 minutes after injection ($a_{15\ min.}$) They compared t_{max} and t_G with their clinical results but also these parameters relatively often gave false negative renograms (25 %) as well as false positive renograms (27 %) When they used a_{max} as the only parameter 45 per cent proved false negative and 47 per cent false positive According to Farmelant et al (1964) and Wax & McDonald (1964) this high percentage of false negative and positive renograms in Stewart and Haynie's (1962) series was due to their not having compared one kidney with the other of the same patient but with normal control values

Farmelant et al (1964) analysed renograms with the retention quotient given under point 3 which they used as an index between the kidneys They found a difference of 20 per cent or more in this retention quotient in patients with known renal arterial stenosis They compared their parameters with t_{max} t_G the quotient between the amplitude of maximum height and the ratio between the activity on each side after half a minute ($a_{30\ sec}$) The use of t_{max} gave correct results in 55 per cent t_G in 83 per cent a_B in 72 per cent and $a_{30\ sec}$ in 56 per cent

Wax & McDonald (1964) used four parameters (t_{max}) maximum height of the curve (a_{max}) the ratio between 15 min value of the curve and the maximum height ($a_{15\ min}$) and the angle to maximum height of the curve i e the angle between the second segment and the base line as measured with a protractor To evaluate any disparity the left value was compared with the right one This evaluation method gave 24 per cent false positive and 7 per cent false negative When they used the criteria of Farmelant et al (1964) 10 per cent were false positive and 48 per cent false negative Wax & McDonald (1964) recommended the use of the renogram as a screening test to eliminate the majority of hypertensive patients in whom renovascular disease was not the cause of their hypertension With their method they detected 93 per cent of patients with renovascular hypertension

Other investigators have reported fairly good results with the use of an index between the kidneys or differences between the kidneys for evaluation of disparity but the series were

Witcofski et al (1961) They used a technique with a short time constant and a rapid recorder and used three time intervals during the initial segment This analysis of the first part of the curve was combined with determination of half-times of the second and the third segments, according to the principles given above

5 *Other methods used*

A number of methods involving more complicated analytical procedures have also been used Thus, Poker et al (1960) plotted the [131]I Diodrast renogram curves on arithmetic paper and evaluated the heights of, and areas under, the three segments of the curve Krueger et al (1961) used a method, according to which the amplitudes were determined at the end of the first segment (a_B) and the amplitude of the maximum height (a_{max}), after which 100 (a_{max} —a_B) was determined as a measure of the total concentration The total concentration was divided by time during the first segment of the curve and the time during which the curve returned to a value equal to the height of the first segment The minute concentration respectively minute excretion were calculated from these data

Hirakawa & Corcoran (1963) proposed a formula based on measurements of the first segment (a_B), the height of the second segment (a_{max}) and the 15 minute level ($a_{15\ min}$) after which they used a formula

$$\frac{(a_{max} - a_B)^2 + (a_{max} - a_{15\ min})^2}{a_{max}{}^2}$$

Attempts have also been made to use a more theoretical analytical evaluation of the "true renal curve" by subtraction of the blood background curves (Horst et al 1961, Magnusson 1962, Tauxe et al 1962), and more extensive mathematical analysis, compartment analysis (Blaufox et al 1963 c, Spencer & Sigman 1963, Coe & Burke 1964, Kutka et al 1964)

Spencer et al (1961) reported a systematic analysis of various parameters calculated from the [131]I Diodrast renogram They found certain slopes and ratios of amplitudes to give the best reproducibility Their results showed that calculations made simply on the basis of amplitudes of the curve were difficult to control *inter alia* because of variation of the detector and kidney geometry

Pircher et al (1963) also found that individual differences in thickness of the absorbing tissue and the distance between the detector and the kidney made it impossible to use amplitudes Despite these objections Pircher et al (1963) recommended maximum height count (a_{max}) the count 15 minutes after injection ($a_{15\ min}$) and t_{max} which they found superior in the analysis of 14 parameters and difference of the parameters between the left and the right kidney Clinically however, several groups have used amplitudes and reported good agreement between such values and their clinical data (Tauxe et al 1962 Burbank et al 1963 Doig et al 1963 Pedersen et al 1964)

A number of other groups have however questioned the value of this

may be measures of the same function. Two parameters calculated from the second and third segment might be sufficient to evaluate the renal curve. Parameters such as ratios calculated from the second segment for the evaluation of uptake function or from the third segment for estimating elimination of the substance would probably be the most suitable for clinical work.

Choice of parameters of the ^{131}I-Hypaque renogram

Fig. 19 a shows an original ^{131}I Hypaque renogram. The renogram shows a rapid initial rise of activity 10—20 seconds after the injection of radioactive Hypaque. This initial upswing is followed by a further ascending segment which rises less rapidly than before to reach a maximum value at 2.5—6 min. after the injection. This is followed by a descending segment with a gradually decreasing disappearance rate from the maximum value to about 20—30 min. During the rest of the examination the activity gradually disappeared at a constant rate. The height of the initial rapid upswing is often difficult to determine with precision because of its continuity with the next segment.

Fig. 20 gives a schematic diagram of the normal ^{131}I Hypaque renogram showing the time of injection and the time at which the two parameters are calculated. As a measure of uptake the ratio between the value at 3.5 min. and that at 1.5 min. were calculated from the second segment of the curve. The ratio between the value at 15 min. and at 30 min. after the injection was used as a measure of the elimination of the substance (excretion ratio I). It is important that these two points are not close to maximum value, but they should not be too late in the curve. The choice of an early value of the curve involves the risk of an increased effect of uptake function which is still going on, while a late value increases the risks of an effect of the activity from the bladder. The graphical description of the renogram is done in an arithmetic scale and all the values are corrected for background activity.

In the acute urinary obstruction series (series II, table XVIII) the excretion ratio (II) was calculated as the ratio between the 5 minute value and the 15 minute value, because in these cases most of the curves could only be recorded for a short period.

Fig. 19 b shows an original external blood curve measured over the chest. The values at 5 and 30 minutes were used to form a ratio which were multiplied by 100 to give a measure of the retention per cent of the isotope.

Theoretical analysis of the renogram and the parameters used

In the analysis of the different factors forming an integral part of the complex external renal curve reference is made to a schematic model of the passage of the test substance through the kidney (Fig. 21). The detector over the kidney records the net effect of simultaneous processes, i. e., the arrival of

smaller than those referred to above (Doig et al 1963, Hirakawa & Corcoran 1963, Wedeen et al 1963, Krogsgaard & Friis 1964, Pedersen et al 1964)

Summing up, though the above mentioned investigators reported varying results of their evaluation procedures, it is generally recognised that the renogram should be evaluated by standard parameters Simple parameters calculated from the second or third segment have been used in the evaluation of the individual curves and for demonstrating disparity between the two kidneys More complicated procedures have also been tried for recognising any difference between the two kidneys in a given patient, but such methods are laborious and are therefore not really satisfactory for clinical use

The Author's method for evaluation of ^{131}I-Hypaque renogram

Evaluation of renograms by visual inspection and from the value of t_{max} has proved less satisfactory (Denneberg & Hedenskog 1959)

Visual inspection is, however, sufficient to demonstrate or exclude urinary obstruction (Denneberg 1959/60) t_{max} in combination with a ratio between the maximum value and the value 30 minutes later implied a considerable improvement (Denneberg 1962) This procedure was not really satisfactory either because it did not allow any definite estimation of uptake function In studies on cats with experimental hydronephrosis ratios were calculated from the 2 and 24 minute values of the renogram as a measure of renal uptake (Denneberg et al 1961 b) The purpose of this ratio was to facilitate comparison between the different curves after various intervals of unilateral ureteric occlusion and to make it possible to follow the course of the impaired renal function

Experimental studies and clinical experiences have shown that the first segment of the renogram is less suitable for the calculation of the parameters, it is too complex and does not properly reflect the vascular capacity of the kidney Another drawback is that the vascular capacity is estimated from a single amplitude and not from a ratio Such amplitudes proved unsuitable because of their dependence on the radioactive dose and sources of error due to the kidney detector geometry In the interpretation of the renogram it was desired to obtain parameters which were independent of the above mentioned factors and which could be calculated for example as a ratio between two amplitudes These ratios should be simple and easy to calculate and it should be possible to use their primary values in the original curve The information obtainable from such parameters should also give a satisfactory description of the changes in the curve due to changes in renal function Thus the information obtainable from the curve does not vary with the number of parameters used As exemplified above (point 3 page 63) several parameters

reaches its maximum there is equilibrium *between inflow and outflow* When the outflow *i e* elimination of the substance is predominant the curve falls It should however be underlined that the latter term is to be understood as elimination *from* the field of vision of the crystal and is not a measure of excretion from the renal pelvis

With the aid of two parameters calculated as ratios from the rising and falling segments of the curve it is possible to obtain four combinations of *theoretical kidney* curves corresponding to different types of disturbed function These curves are exemplified in Fig 22 Type A has a normal rate of inflow and outflow while B represents a curve with decreased rate of inflow but normal outflow This curve shows decreased uptake capacity with an abnormally slow rise which causes a delay of the maximum value of the curve The excretory segment shows a slower decline because uptake of the substance persists longer than normal Is This means that a ratio calculated from this segment decreases with impairment of uptake function even if outflow is not obstructed Type C shows a normal inflow but decreased outflow while Type D shows both decreased inflow and outflow

Fig 23 gives a theoretical survey of the expected relation between uptake and *excretion ratio* The figure shows how the two ratios should be for the four different types of curves which are given with their numbers in separate fields Type A is located in the field with normal uptake and *excre*

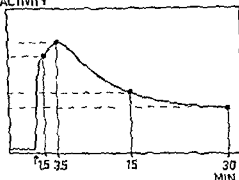


FIGURE 20 Reference points used in the analysis of ^{131}I Hypaque renogram in the present investigation Arrow Time of injection

tion ratio while type B shows (depending on the severity of the impairment of uptake) a successive movement from the upper right field to the lower left on the diagram along the broken line

That none of the ratio values are within the lower right field is explained by the fact discussed above that despite free drainage the excretion ratio is decreased in kidneys with impaired uptake capacity The example shows the close dependence of excretion ratio on uptake ratio from which it cannot be judged separately Type B as a final stage lies lowest in the

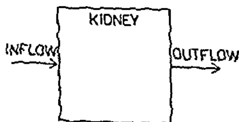


FIGURE 21 Schematic model of passage of *test substance* through the kidney

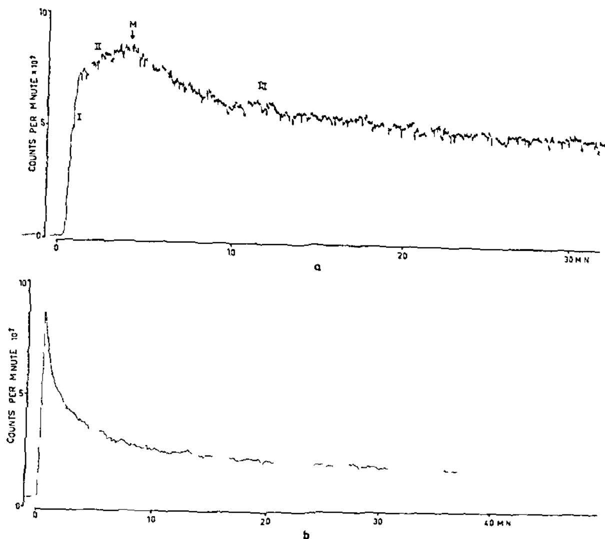


FIGURE 19 a Original ^{131}I Hypaque renogram in a control O Time of injection I 1st segment. II 2nd segment III 3rd segment M Maximum height b Original Hypaque external chest curve

the substance and its accumulation in the kidney (inflow) and its elimination from the kidney (outflow) Inflow thus largely represents the renal circulation of the substance its glomerular filtration and passage via peritubular capillaries into the tubular cells and out to the lumina of the tubuli, *i e* all processes contributing to accumulation of the substance in the kidney As long as inflow is predominant, the curve will rise The other processes during the further intrarenal passage through the various parts of the nephron (convoluted and collecting ducts) down to the renal pelvis and the simultaneous reabsorption of water on the other hand produce no increase in the activity and therefore no further rise of the curve The detector cannot distinguish these simultaneous kinetic processes but records only the sum of renal and extrarenal activity When the curve

reaches its maximum there is equilibrium between inflow and outflow. When the outflow, i.e. elimination of the substance is predominant, the curve falls. It should however be underlined that the latter term is to be understood as elimination from the field of vision of the crystal and is not a measure of excretion from the renal pelvis.

With the aid of two parameters calculated as ratios from the rising and falling segments of the curve it is possible to obtain four combinations of theoretical kidney curves corresponding to different types of disturbed function. These curves are exemplified in Fig. 22. Type A has a normal rate of inflow and outflow, while B represents a curve with decreased rate of inflow but normal outflow. This curve shows decreased uptake capacity with an abnormally slow rise which causes a delay of the maximum value of the curve. The excretory segment shows a slower decline because uptake of the substance persists longer than normally. This means that a ratio calculated from this segment decreases with impairment of uptake function, even if outflow is not obstructed. Type C shows a normal inflow but decreased outflow, while type D shows both decreased inflow and outflow.

Fig. 23 gives a theoretical survey of the expected relation between uptake and excretion ratio. The figure shows how the two ratios should be for the four different types of curves, which are given with their numbers in separate fields. Type A is located in the field with normal uptake and excretion ratio, while type B shows (depending on the severity of the impairment of uptake) a successive movement from the upper right field to the lower left on the diagram along the broken line.

That none of the ratio values are within the lower right field is explained by the fact discussed above that despite free drainage the excretion ratio is decreased in kidneys with impaired uptake capacity. The example shows the close dependence of excretion ratio on uptake ratio from which it cannot be judged separately. Type B is a final stage, lies lowest in the

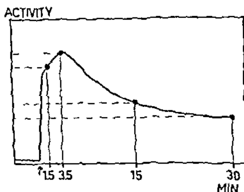


FIGURE 20. Reference points used in the analysis of ¹³¹I Hypaque renogram in the present investigation. Arrow: Time of injection.

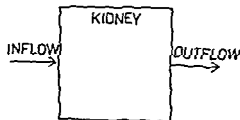


FIGURE 21. Schematic model of passage of test substance through the kidney.

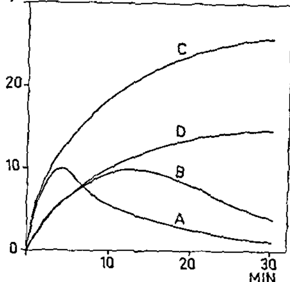


Figure 22 Theoretical kidney curves for different types of disturbed function (A B C and D see page 69)

left field, and the curve is then identical with a body background curve

In principle one might imagine a type of curve localised in the field to the right above the normal field (marked with broken line and arrow) This curve represents abnormally high uptake with a normal excretion ratio and might correspond to a hypertrophic kidney Type C is localised in the upper left field and shows decreased excretion ratio with normal uptake ratio This would correspond to the type of curve seen in acute urinary obstruction On removal of the obstruction it would return to the normal field The last type (D) corresponds to impaired uptake (decreased uptake ratio) in combination with decreased excretion This curve moves towards the lower left field where the uptake has ceased and corresponds to a nonfunctioning kidney in the final stage

It should be stressed that these types of curves are based on theoretical renal curves and not on true renograms

One must also consider factors outside the kidney, e g effect of extrarenal activity (Fig 24) The figure shows the time curves of the distribution of ^{131}I Hypaque in the organism after an intravenous injection P represents the blood activity, P + E the sum of the activity in the blood and extravascular space and U the cumulative excretion to the bladder During the ascent and descent of the kidney curves, curve P shows a successive fall in blood activity and curve U, after an initial delay, rises as a sign of continued accumulation of radioactivity in the bladder A substance with high clearance rate e g ^{131}I Hippuran, has a steeper second segment and the dec

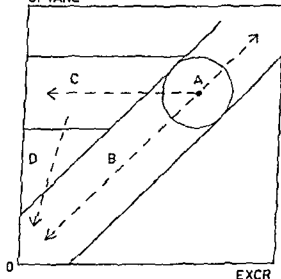


Figure 23 Theoretical survey of expected relation between uptake and excretion ratio A Normal function B Decreased rate of inflow C Decreased rate of outflow D Decreased rate of inflow and outflow

line of the third segment is more marked than for substances with lower clearance rate e g ¹³¹I Hypaque This is reflected also in curve P by a more rapid decline in the concentration and in curve U by a more rapid rise of the bladder curve

The effect of the non renal factors on the renogram can thus complicate the use of the parameters Despite this objection it is of interest to ascertain whether parameters can be calculated from the more complex renogram seen in different types of renal impairment to test the schematic renal model, and to find out to what extent theoretical analysis of kidney curves agrees with the renograms used in clinical practice The results of the renograms can be illustrated by a similar diagram with the two parameters plotted against each other

Figs 25 a and b are based on 173 and 182 kidneys from series A—I for comparison with different types of functional disturbances An account is given of four respectively five groups including normal kidneys kidneys with known urinary obstruction (acute or chronic) kidneys from patients with other renal diseases without known urinary obstruction and finally a group with aplasia status post nephrectomy or a silent kidney Verification of the presence or absence of urinary obstruction was obtained by urography and/or retrograde pyelography renal angiography or post mortem examination

It is clear from Figs 25 a and b that the distribution of the various function groups among separate fields

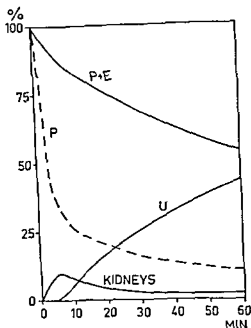


FIGURE 21 Schematic survey of fate of Hypaque after single injection P Plasma curve P + E Sum of plasma and extra vascular tissue U Cumulative urinary excretion.

was largely the same as that in the theoretical diagram (Fig 23) The borders of the fields of these groups were based on observed values Fields with normal uptake and excretion ratio occupied corresponding positions (I and A) and this holds also for the fields with decreased uptake and normal excretion ratio (II and B) The two fields C and D in the theoretical diagram on the other hand show only one field (III) in which the series with verified urinary obstruction (acute or chronic) are localised In Fig 25 the upper right part contains a field above the normal field (IV) The former includes cases with an abnormally high

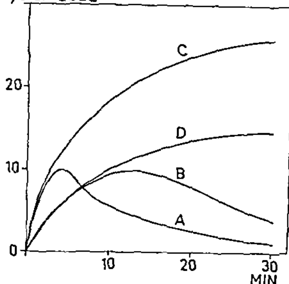


Figure 22 Theoretical kidney curves for different types of disturbed function (A B C and D see page 69)

left field, and the curve is then identical with a body background curve

In principle one might imagine a type of curve localised in the field to the right above the normal field (marked with broken line and arrow) This curve represents abnormally high uptake with a normal excretion ratio and might correspond to a hypertrophic kidney Type C is localised in the upper left field and shows decreased excretion ratio with normal uptake ratio This would correspond to the type of curve seen in acute urinary obstruction On removal of the obstruction it would return to the normal field The last type (D) corresponds to impaired uptake (decreased uptake ratio) in combination with decreased excretion This curve moves towards the lower left field where the uptake has ceased and corresponds to a non functioning kidney in the final stage

It should be stressed that these types of curves are based on theoretical renal curves and not on true renograms

One must also consider factors outside the kidney, *e g* effect of extrarenal activity (Fig 24) The figure shows the time curves of the distribution of ^{131}I-Hypaque in the organism after an intravenous injection P represents the blood activity, P + E the sum of the activity in the blood and extravascular space and U the cumulative excretion to the bladder During the ascent and descent of the kidney curves, curve P shows a successive fall in blood activity and curve U, after an initial delay, rises as a sign of continued accumulation of radioactivity in the bladder A substance with high clearance rate, *e g* ^{131}I Hippuran has a steeper second segment and the dec

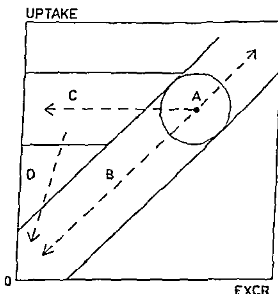


Figure 23 Theoretical survey of expected relation between uptake and excretion ratio A Normal function B Decreased rate of inflow C Decreased rate of outflow D Decreased rate of inflow and outflow

G B (acute ureteral obstruction) shows the curve seen in urinary obstruction with normal uptake but decreased excretion ratio Type IV (case L A) illustrates the type seen for renal hypertrophy with abnormally large uptake and with normal excretion ratio

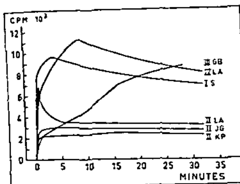


FIGURE 26 Various types of ¹³¹I Hypaque renograms in 5 cases. The Roman numbers indicate fields given in Fig. 25 a Case S C (Series A) Control (I) Case K P (Series D) Congenital multiple cystic kidney (II) Case J G (Series F) Nephrosclerosis (II) Case L A (Series G) Right renal aplasia (II) and left kidney enlarged (compens. hypertrophy) (IV) Case G B (Series H) Acute ureteral obstruction. (III)

D Results

Controls (Series A)

Table XV gives the patients initials sex age the three renogram parameters (uptake excretion ratios I and II) of the kidneys retention per cent calculated from the chest curve N P N and the diagnoses Statistical analysis with the Students t test showed that the differences between the respective parameters (right minus left) did not differ significantly from zero ($P > 0.05$) i e there was no significant difference between the sides compared regarding uptake or the two excretion ratios The normal limits were therefore determined for the pooled series irrespective of the side Owing to the skewed distribution of uptake respectively excretion ratio I the mean value (M) and the standard deviation (SD) were determined from the logarithmic values of the series In the determination of the corresponding values for excretion ratio II the original values were used because the distribution of this material was normal Since no significant difference was found between the two sides the two renograms were directly comparable with one another and any difference between them may be ascribed to differences in renal function

Criteria of normal ¹³¹I Hypaque renogram

A Hypaque renogram was accepted as normal when the values of both parameters were within the normal limits of the control series The mean uptake ratio was 1 075 and M ± 2 SD 1 017—1 131 Corresponding values for the excretion ratio I were 1 194 with a range of 1 085—1 316 and for excretion ratio II 1 288 respectively 1 087—1 490

The normal range of the excretion ratio II was wider than that of excretion ratio I This is because excretion ratio II is a less true measure of elimination because it measures a considerable component of the uptake

uptake ratio, i.e., the type of curve seen in renal hypertrophy, which is dwelt on in the chapter on the results (chapter VIII D). The agreement found between the parameters calculated from the theoretical curves and the more complicated renograms show that the method of evaluation can be used in clinical practice.

Fig. 26 shows different types of renograms from the clinical series, which correspond to the figures given in the fields. Type I (case S. G.) is a normal curve. Type II includes three variants of curves representing different degrees of impaired uptake function. The uppermost curve does not show any uptake segment, but only a slowly filling excretion segment, i.e. the type seen in non functioning kidneys (case L. A., renal aplasia). The other two curves show decreased uptake ratio, which influences the excretion ratio and also causes a delay of the maximum value of the curve (cases J. G. and K. P.). Type III (case

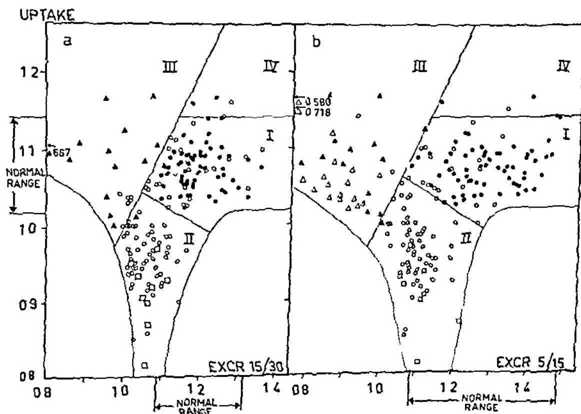


Figure 25 a. Uptake ratio plotted against excretion ratio 15/30 (I) in different types of functional disorders in 173 kidneys of series A—I except series D and H. Demarcation lines of the fields (I—IV) based on observed values. Solid circles: Control kidneys. Open circles: Kidneys with various diseases without signs of obstruction. Solid triangles: Kidneys with signs of chronic obstruction. Open squares: Aplasia, post nephrectomy or silent kidney. b. Uptake ratio plotted against excretion ratio 5/15 (II) in different types of functional disorders in 182 kidneys of series A—I except series D. Open triangles: Kidneys with signs of acute ureteral obstruction. Other symbols as in Fig. 25 a.

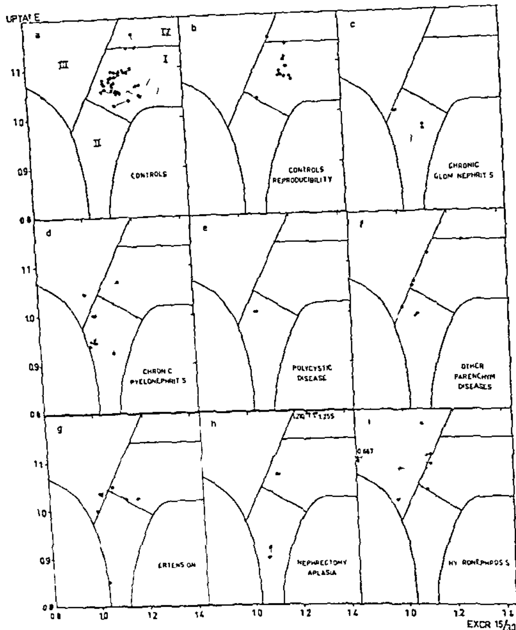


FIGURE 27. Uptake ratio plotted against excretion ratio 15/30 (I) in series A–G and I. Ratio values in individual cases joined by lines. The Roman numbers indicate fields given in Fig. 5a.
a and b Series A. c Series B. d Series C. e Series D. f Series E. g Series F. h Series G. i Series I. 5 cases in series C excluded because of signs of obstruction and 4 cases in series B–E for technical reasons.

function with a consequently wider scatter of this parameter As discussed in Chapter VIII B, however, the choice of excretion ratio II was dictated by the conditions prevailing at the time of the examination

Disparity between the two external renograms was said to exist when the differences between the respective parameters (right minus left) were larger than those observed in the control group (Fig 29) In Fig 29 the difference is given irrespective of sign

Reproducibility

The reproducibility of the method was checked by determinations at two days interval in ten controls The results (given in Table XVI and Fig 27 b) showed no significant difference between the parameters calculated from the renograms (uptake and excretion ratio I) The error of the method (S E) of the uptake ratio was found to be 2 7 per cent, while the corresponding error for the excretion ratio I was 2 7 per cent

Renal disease series (B—I) Analysis of uptake and excretion ratio

Tables XVII—XIX show the cases in the various series of renal diseases with regard to initials sex age, the three renogram parameters (uptake and excretion ratios I and/or II) of the kidneys retention per cent calculated from the chest curve and laboratory findings (N P N, serum creatinine creatinine clearance maximal specific gravity) and diagnoses The cases in the series with parenchymal renal disease including hypertension, are arranged according to the severity of impairment of renal function The acute obstruction series is divided into two groups according to whether the diagnosis was roentgenologically verified or not

Figs 27 a—i and 28 a—c give the uptake by each kidney and the excretion ratio (I respectively II) in the control series and in the various series of renal diseases Five cases were not included in the series for technical reasons (V B, L L, K J, K K and A B) The limits of the four fields discussed above (page 71) are inserted in each figure To compare the values for the two sides in individual patients the two values have been joined

Field I in Fig 27 a—i shows the values for controls and for patients with normal values in the other series The latter represent half of the cases in the series with hypertension and other parenchymal disease while the majority of the remainder in these series fall within field II Field II comprises all the cases with chronic glomerulonephritis and the majority of kidneys in the series of chronic pyelonephritis and polycystic kidneys and in the lower part of the field cases with aplasia and status post nephrectomy or silent kidneys The poorer the uptake the lower the position of the ratio in the field There is good agreement between the position due to changes in uptake ratio of the individual kidneys and function as judged from creatinine clearance serum creatinine and N P N It is noteworthy that the series of *aplasia or status post*

horizontal difference was found between the sides and as mentioned above the pathological obstructed side fell within field III (Fig. 27 a). The contralateral kidney fell within the normal field or sometimes in field II. This field also comprises three of the patients with hydronephrosis where the contralateral kidney was functionless or had been removed.

Figs. 28 b and c where the uptake ratio is compared with the excretion ratio 5/15 (II) show the same distribution of kidneys with obstruction as that of kidneys with hydronephrosis in Fig. 27. The majority of the contralateral kidneys in the patients with unilateral urinary obstruction fell within the normal field. The remaining cases in this series with non verified urinary obstruction showed small differences and the majority of them were situated within fields I or II. One exception in this latter series was case M. J. who had undergone nephrectomy and in whom the remaining kidney was normal which explains the considerable difference between the sides.

Fig. 29 shows the difference between the right and left kidneys regarding uptake and excretion ratios I and II in all series (A—I). Nine of the cases in the series parenchymal renal disease including hypertension showed abnormally large differences. Eight of the cases differed in uptake ratio while five of the nine also differed in excretion ratio. Of these nine cases three belonged to series C (cases B. M., B. Z., A. E.), two to series D (cases K. P., Z. N.), two to series E (cases H. I.,

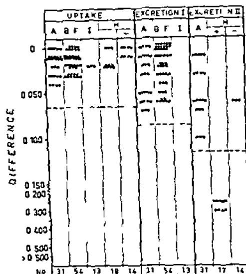


FIGURE 29. Difference between right and left renogram parameters in series A—I. Series H is divided into two groups with (+) and without signs of obstruction (—). 2 cases excluded for technical reasons (Series H).

K. M.) and two to series I (cases B. F., I. J.).

In series H the difference in excretion ratio II clearly distinguished the roentgenographically verified cases with obstruction from those where roentgen examination revealed no obstruction. Only case C. N. fell within the limits of the control series and that patient was one of those who could not sit still during the test because of pain (C. N., A. B., E. R. and N. W.). In case A. B. the renogram was less satisfactory because after eight minutes recording the patient vomited and had severe pain and then moved from the detector over the left kidney. The excretion ratio (5/15) could therefore not be calculated. The patient was therefore excluded in the calculation

nephrectomy includes c ises where the i itio for the contralateral kidney is situated in field IV In these cases the roentgen examination verified com pens itory hypertrophy of the kidney Though the number of these cases is small, this localisation appears to con firm the validity of the previously described theoretical analysis Field III in Figs 27 a and 28 b comprises the cases with urinary obstruction, i e with normal uptake but decreased ex cretion ratio The m ijority of the con tralateral kidneys in these series with out obstruction lay within field I or II, as did cases in the unverified cases of acute obstruction (Fig 28 c)

Disparity between left and right renograms

As shown in Fig 27 c—h the series with parenchym il ren il dise ises in cluding hypertension and apl isia or st itus post nephrectomy differed m ini ly along the vertical axis

In the series chronic glomerulone phritis there was only a sm ill differ ence between the v ilues comp ired, while in the series chronic pyelo nephritis, polycystic kidneys, other p irenchym il disease and the series with hypertension the disp irity w is occ ision illy considerable In these cases with disp irity between the sides the "he ilthy" side usu illy fell within the norm il field I while the iffected side w is situ ited in field II Extreme differences were, of course noted in the series aplasi i and st itus post nephrectomy, where the remaining kidney fell within the normal field or the field for hypernorm il upt ike r itio The v ilue over the site of the removed kidney fell within the lower p irt of field II, i e a sign of no upt ike

In the series with obstruction a

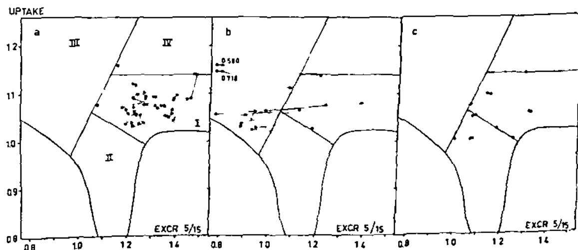


Figure 28 Uptake ratio plotted against excretion ratio 5/15 (II) in series A and II Ratio values in individual cases joined by lines The Roman numbers indicate fields given in Fig 26 b

a Controls (Series A) b Cases with signs of obstruction c. Cases without signs of obstruction (Series II) One case in series II excluded for technical reasons

horizontal difference was found between the sides, and as mentioned above the pathological obstructed side fell within field III (Fig. 27 a). The contralateral kidney fell within the normal field or sometimes in field II. This field also comprises three of the patients with hydronephrosis where the contralateral kidney was functionless or had been removed.

Figs. 28 b and c where the uptake ratio is compared with the excretion ratio 5/15 (II) show the same distribution of kidneys with obstruction as that of kidneys with hydronephrosis in Fig. 27. The majority of the contralateral kidneys in the patients with unilateral urinary obstruction fell within the normal field. The remaining cases in this series with non-verified urinary obstruction showed small differences and the majority of them were situated within fields I or II. One exception in this latter series was case M. J. who had undergone nephrectomy and in whom the remaining kidney was normal, which explains the considerable difference between the sides.

Fig. 29 shows the difference between the right and left kidneys regarding uptake and excretion ratios I and II in all series (A—I). Nine of the cases in the series parenchymal renal disease including hypertension showed abnormally large differences. Eight of the cases differed in uptake ratio while five of the nine also differed in excretion ratio. Of these nine cases three belonged to series C (cases B. M., B. Z., V. I.), two to series D (cases K. P., Z. N.), two to series I (cases H. J.

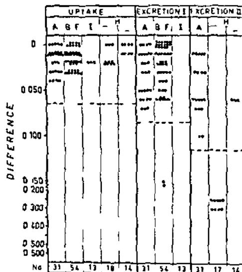


FIGURE 29. Difference between right and left renogram parameters in series A—I. Series II is divided into two groups with (+) and without signs of obstruction (—). 2 cases excluded for technical reasons (Series II).

K. M.) and two to series I (cases B. J., I. J.).

In series II the difference in excretion ratio II clearly distinguished the roentgenographically verified cases with obstruction from those where roentgen examination revealed no obstruction. Only case C. N. fell within the limits of the control series and that patient was one of those who could not sit still during the test because of pain (C. N., A. B., I. R. and N. W.). In case A. B. the *renogram* was less satisfactory because after eight minutes recording the patient vomited and had severe pain and then moved from the detector over the left kidney. The excretion ratio (6/1a) could therefore not be calculated. The *patient* was therefore excluded in the calculation

nephrectomy includes cases where the ratio for the contralateral kidney is *situated in field IV*. In these cases the roentgen examination verified compensatory hypertrophy of the kidney. Though the number of these cases is small, this localisation appears to confirm the validity of the previously described theoretical analysis. Field III in Figs. 27 a and 28 b comprises the cases with urinary obstruction, *i.e. with normal uptake but decreased excretion ratio*. The majority of the contralateral kidneys in these series without obstruction lay within field I or II, as did cases in the unverified cases of acute obstruction (Fig. 28 c).

Disparity between left and right renograms

As shown in Fig. 27 c—h the series with parenchymal renal diseases including hypertension and aplasia or status post nephrectomy differed mainly along the vertical axis.

In the series chronic glomerulonephritis there was *only* a small difference between the values compared, while *in the series* chronic pyelonephritis, polycystic kidneys, other parenchymal disease and the series with hypertension the disparity was occasionally considerable. In these cases with disparity between the sides the "healthy" side usually fell within the normal field I, while the affected side was situated in field II. Extreme differences were, of course, noted in the series aplasia and status post nephrectomy, where the remaining kidney fell within the normal field or the field for hypernormal uptake ratio. The value over the site of the removed kidney fell within the lower part of field II, *i.e.* a sign of no uptake.

In the series with obstruction, i

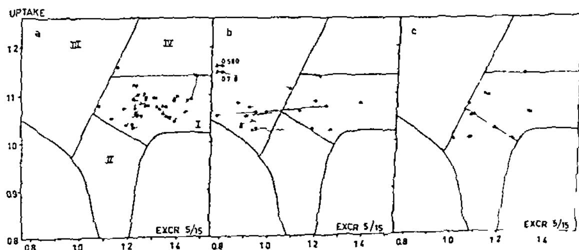


FIGURE 28. Uptake ratio plotted against excretion ratio 5/15 (II) in series A and II. Ratio values in individual cases joined by lines. The Roman numbers indicate fields given in Fig. 25 b.
a Controls (Series A). b Cases with signs of obstruction. c Cases without signs of obstruction (Series II). One case in series II excluded for technical reasons.

Table VII Comparison between urograms and renograms in cases of renal parenchymal diseases and hypertension (Series B—F)

Excretion urography	Renography		
Findings	Normal on both sides	Disparity between sides[1]	Both sides abnormal (no disparity)
Normal	3	0	7
Changes on one side	3	8	1
Changes on both sides	1	0	10

[1] Unilateral changes in all cases

Table VIII Comparison between findings made at roentgen examination laparotomy autopsy and renography (Series B—I)

Roentgenography	Renography		
Findings	Normal on both sides	Disparity between sides[1]	Both sides abnormal (no disparity)
Normal	3	0	5
Changes on one side	3	8	1
Changes on both sides	1	1	22

[1] Unilateral changes in 8 and bilateral in 1

aminations and laparotomy provided the possibility of judging the morphological changes including differences in kidney size While the roentgen examination and autopsy thus allowed estimation from a qualitative point of view the renogram allowed evaluation from a quantitative functional point of view The roentgenographic and autopsy findings were divided into three groups (1) normal (2) changes on one side and (3) changes on both sides The renograms were classified as (1) normal on both sides (2) disparity between the sides (unilateral or bilateral abnormality) and (3) both sides abnormal (no disparity)

33 patients were examined with urography and renography (Table VII) and in 21 the findings made by both examinations were in good agreement Corresponding figures in Table VIII show 44 cases examined of which agreement was found in 33 To elucidate the cause of the difference between the urography and renography the findings were compared with the results of renal function tests (creatinine clearance serum creatinine N P N)

of the difference in excretion ratio in Fig 29, but is given as a roentgenographically verified case of obstruction in the other diagrams for the right side because the curve for this side ascended continuously without showing any maximum value during the measuring time

The difference in uptake ratio on the two sides for the corresponding series showed that cases E R, A B and N W fell outside the limits of the control series as well as case M J with aplasia (not marked in Fig 29) The values for the remaining 15 cases with, and 14 without, roentgenographically verified obstruction fell within the limits of the control series

In three of the thirteen roentgenologically verified cases of hydronephrosis in series I the difference in excretion ratio 15/30 fell within the limits of those of the control series One (S A) of the cases, however, had roentgenographically verified bilateral hydronephrosis Cases E H and I W had unilateral pathological curves on the roentgenographically verified pathological side But the difference between the two sides lay within the limits of the control series In five of the cases the difference in uptake ratio was abnormally large In one case the difference could be explained by a tumour of the pelvis which had caused extensive destruction of the renal parenchyma (case I W) In another case (S A) with bilateral hydronephrosis, it had proved necessary to reimplant the left ureter after the kidney had been shut off for a long time following an operation because of gynaecological cancer In the remaining cases the difference between the sides could be explained by angiographically verified silent kidneys (cases E H and F J) and in the third (I N) the patient had undergone nephrectomy

Comparison between findings made at excretion urography, laparotomy or post mortem examination and renography

In four cases (V B, L L, K J, K K) no comparison was made between the sides because of technical errors of the renograms on one side

The number of roentgen examinations in each series of renal disease (B—I) is given in Table VI Table XX summarises the results of the roentgen examination and post mortem examination together with the findings at laparotomy

Table VII compares the results of excretion urography and radioisotope renography and Table VIII the findings made at roentgenography, post mortem examination and laparotomy with those at renography The cases in Tables VII and VIII consist of series B—I, i e parenchymal renal diseases including hypertension The urograms were judged with respect to excretion time of contrast medium density of contrast medium morphological changes and significant differences in size between the two kidneys In some cases excretion urography was supplemented by retrograde pyelography selected renal angiography or aortography for verification of the urographic findings Post mortem ex

other renal function tests An exception was case V O in whom function tests gave normal values but who showed clinical signs of renal injury with proteinuria microhaematuria and pathological quantitative sediment In the other six patients the creatinine clearance and/or serum creatinine and/or N P N were abnormal

In the last case (R M) urography showed unilateral tuberculous changes which were verified at operation The renogram showed bilateral changes but the serum creatinine and the N P N were normal

The majority of the new cases in Table VIII were patients examined at autopsy They have not been included in Table VII because the excretion urography could not be done owing to impairment of renal function The causes were chronic glomerulonephritis or pyelonephritis or nephrosclerosis All had bilateral changes on the basis of their renal diseases All 22 cases in the series except K S had pathological values for one of the three function tests creatinine clearance serum creatinine and N P N (19/21 19/22 respectively 16/22)

In case Z N who died from a ruptured aortic aneurysm post mortem examination showed bilateral polycystic kidneys with preserved parenchyma on both sides and without any certain morphological difference between the sides The renogram showed bilateral changes but also disparity The bilaterally abnormal renograms corresponded to a decreased creatinine clearance of 72 ml/min and increased serum creatinine of 1 30 mg/100 ml and N P N of 41 mg/100 ml but no explanation of the functional difference could be obtained

Comparison between uptake ratio and other renal function tests with radioactive Hypaque

Fig 30 a—h gives the uptake ratio for the symmetric renograms (curves without disparity) calculated as the mean of the right and left sides This provides a possibility of obtaining measures comparable with those of other tests of total renal function The same procedure was adopted in cases with asymmetric renograms but in Fig 30 a—h these cases are marked (solid circles) since this procedure cannot be regarded as really correct because of the differences between the function of the two kidneys However most of these cases show no noteworthy deviation from the others

Fig 30 a—d shows that despite considerable spread of the values in several of the series there was a clear linear relation between the uptake ratio and renal function data obtained with Hypaque The coefficient (r) of correlation between uptake ratio and clearance with intravenous infusion technique was + 0 67 between uptake ratio and 2 hour urinary recovery + 0 60 between uptake ratio and retention per cent calculated from the blood curve — 0 60 and between uptake ratio and retention per cent calculated from the chest curve — 0 71 The correlation between the uptake ratio and the disappearance of ^{131}I Hypaque from the blood (plasma re

In the three cases with normal urograms and renograms renal function was normal with the exception of N N, in whom clearance was slightly decreased (86 ml/min) The eight cases with disparity between the kidneys were identical with those cases shown in Fig 29 where a difference was found either in the uptake or excretion ratio or in both The ninth case was Z N, who was not examined with urography because of decreased renal function The cases could be classified into three groups regarding the difference in uptake and/or excretion ratio The abnormal curves were of those types (I—III) described in Fig 26 All the cases had normal creatinine clearance, serum creatinine and N P N, except one (K M, N P N 44 mg/100 ml) and all had either morphological changes or significant differences in the size of the two kidneys

In all ten cases with bilaterally abnormal renograms but without disparity, except case K S (horseshoe kidneys), the urograms showed poor excretion bilaterally, which corresponded well with bilateral abnormal renograms and with the other renal function tests Of the ten nine had decreased creatinine clearance nine increased serum creatinine and five increased N P N Judging from the renogram, case K S had a normal uptake ratio with decreased excretion ratio, *i e* the type found in urinary obstruction but without a certain disparity between the curves The finding corresponded well to the retarded excretion found at urography and to other normal results of function tests

In cases T Y, Ö N and R A with discrepancies between the urograms and renograms the renograms were normal on both sides, while urography suggested unilateral changes In cases Ö N and R A excretion urography showed differences in the size of the kidneys with normal morphology Supplementary aortography showed a normal vascular pattern Post mortem examination showed no significant difference between the kidneys in these two cases except that R A had a small stone and a local pyelonephritic scar in the right lower pole but no other changes in the renal parenchyma or signs of obstruction

Case Ö N had bilateral nephrosclerosis without any difference between the two sides In the third case (T Y) the urogram showed double pelvis and ureters on the left side a normal rate of excretion and a normal density on both sides Renal function tests gave normal results and the renographic findings were normal

In case H S the renogram was normal and urography showed normal excretion density and morphology but abundant small diffuse calcifications in the renal parenchyma on both sides indicating nephrocalcinosis As expected from the normal renogram the results of renal function tests were normal

In the seven cases where the urographic appearance was normal on both sides while the renogram showed bilateral abnormal curves without disparity there was good agreement between the abnormal renogram and

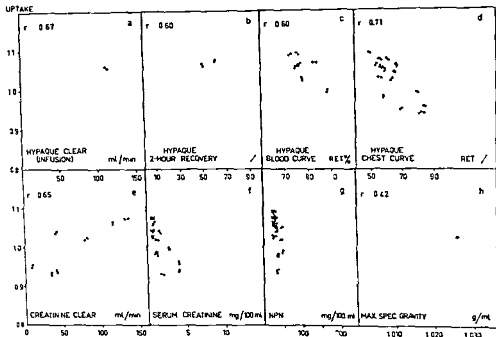


FIGURE 30 a—h Mean value of uptake ratio on both sides plotted against Hypaque clearance with infusion technique (24 cases) 2 hour urinary recovery of Hypaque (17 cases) retention per cent calculated from blood and chest curves (58 and 83 cases) creatinine clearance (48 cases) serum creatinine (53 cases) serum N P N (75 cases) and max spec. gravity (26 cases) in series A—I Solid circles Cases with symmetric renograms Open circles Cases with asymmetric renograms Hypaque and creatinine clearance are corrected to hold for 1 73 m² body surface

curate enough to decide whether the patient had urinary obstruction or not Good results with this technique have been reported by Tauxe et al (1962) and Farschidpur & Schoknecht (1963)

In the investigations a rather wide collimator was used in order to allow a view of the entire kidney This technique enables a more reliable positioning over the region of the kidney than narrow collimation the latter method placing large demands on the examiner s skill (Sälerborg 1960 Johnson et al 1964) Sälerborg (1960) analysed the problem of proper positioning of the detectors in renography with the aid of focusing under fluoroscopic control With very narrow collimation (aperture of 12.5 mm) and varying centering of the detector he obtained different types of curves Proper evaluation of the renogram requires correct centering of the detector over the kidneys especially if such evaluation is based on measurements of amplitudes for then small differences in the position of the detector may have a disproportionate effect on the results

spectively chest curve), its clearance by the kidney and the urinary output showed that the parameter of the second segment of the renogram is a measure of renal function The results also showed good agreement between these "external" and "internal" measurements with the radioactive substance

Comparison between uptake ratio and other renal function tests (creatinine clearance, serum creatinine, serum N P N and maximal specific gravity)

Fig 30 e—h gives the means of the uptake ratio in the two sides plotted against creatinine clearance, serum creatinine serum N P N and maximal specific gravity While the serum creatinine and serum N P N show a non linear relation with the uptake ratio, the creatinine clearance and maximal specific gravity show a linear relation (r = + 0 65 respectively r = + 0 42)

These results thus point in the same direction as the above mentioned ^{131}I Hypaque data i e that the uptake ratio is a useful measure in the evaluation of renal function but allows no estimation of glomerular filtration or tubular secretion separately

Comparison between retention per cent calculated from chest curve and other renal function tests (^{131}I Hypaque clearance, 2 hour urinary recovery of ^{131}I Hypaque, retention per cent calculated from the plasma curve and creatinine clearance, serum creatinine and serum N P N)

Fig 31 a—f shows that the retention calculated from the external chest curve is correlated linearly with the clearance of ^{131}I-Hypaque given by intravenous infusion (r = —0 80), 2 hour urinary recovery of ^{131}I Hypaque (r = —0 74) and retention calculated from the plasma curve of ^{131}I Hypaque (r = + 0 70) A similar relation was also found between creatinine clearance (*r* = —0 75) and a non linear relation with serum creatinine and N P N These correlations show that the retention parameter used is a useful supplement to renography

E Discussion

The positions of the kidneys is determined roentgenographically in films taken with the patients sitting upright or by manual scanning after injection of ^{131}I Hypaque nearly always showed good agreement with the results obtained by control scanning after the test Some investigators have used roentgenography and marking of the sites of the kidneys (Winter 1959 Spencer et al 1961 and others) others scanning of the back after injection of a radioactive test dose before the test (Frohlich et al 1959 Magnusson 1962 Dore et al 1963 Johnson et al 1964)

In the acute urinary obstruction series where manual scanning was used a few of the patients who were in pain moved relative to the detector The examination could nevertheless be performed and the results were ac

effect of the renal collimator was examined showed that the renal detectors were influenced only slightly by radioactivity from the contralateral kidney and the bladder

One of the main questions is how the renogram should preferably be evaluated Before attempting to answer this and related questions it must be decided which segment should be used for quantitative analysis The results of experimental renography were discussed in Chapter VIII C The results suggested that the first segment of the curve was less suitable while the second and third segments could be utilized best in the evaluation of the renal uptake and excretion of the substance For analysis of the complex external renal curve a schematic diagram was given where the theoretical renal curve was said to express the net effect of the processes operating simultaneously in the kidney The processes were summarized as inflow and outflow of the substance from the region of the kidney In order to obtain a quantitative measure of these two processes ratios were calculated from the rising respectively falling limbs of the curve These parameters were presumably normal or decreased and four possible combinations were obtained which represented four different types of renal curves These four types fell into different fields in the theoretical Fig 23 where the two parameters were plotted against each other

When the two parameters were calculated from the renograms in the present material their distribution among the fields was largely the same as the theoretical distribution with the exception that the values for acute and chronic urinary obstruction were not separated The less good agreement between the theoretical requirements and the results obtained regarding this field was due to the fact that the uptake by the kidneys with chronic urinary obstruction was nearly always normal This explains why this series did not differ from the cases in the field with acute urinary obstruction Theoretically in a large series (including cases with reduced function) and with a more sensitive test substance than Hypaque it should be possible to demonstrate the theoretically defined relation with division of the field for urinary obstruction into two parts It is of interest to note that neither the theoretical nor the clinical diagram contained any values in the lower right field where the curves with decreased uptake ratio and normal excretion ratio should have been This is because the excretion ratio is lower in patients with decreased uptake ratio even if the urinary drainage is unobstructed (see page 69)

Fig 25 contained no values in the lower left field It should have contained curves with decreased uptake and excretion ratios The values for excretion ratio were however situated to the right of the axis because the substance was eliminated by the contralateral kidney and gave a curve with a more rapid fall and an artificially high excretion ratio

The results show that the theoretical model with four different types of

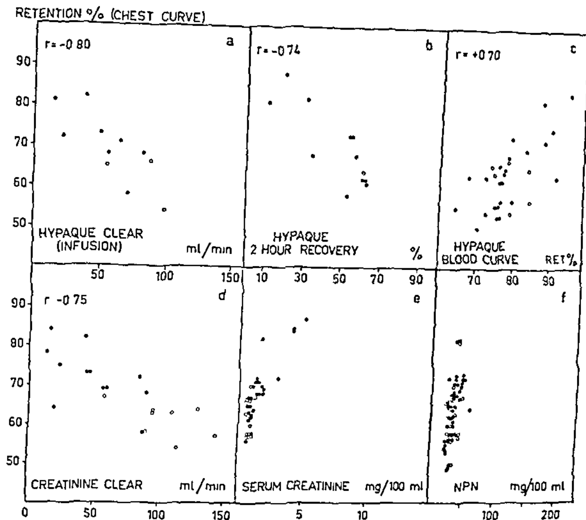


Figure 31 a—f Retention per cent calculated from external chest curve plotted against Hypaque clearance with infusion technique (25 cases) 2 hour urinary recovery of Hypaque (18 cases) retention per cent calculated from blood curve for Hypaque (59 cases) creatinine clearance (57 cases) serum creatinine (63 cases) and serum N P N (88 cases) in series A—F and I Solid circles Cases with symmetric renograms Open circles Cases with asymmetric renograms Hypaque and creatinine clearances are corrected to hold for 1 73 m² body surface

Ratios between two amplitudes are less sensitive to such changes in the position of the detector over the kidney (Spencer et al 1961 Stewart & Haynie 1962, Wax & McDonald 1962 Krogsgaard & Friis 1964) As discussed in Chapter VIII C the lack of a standard technique of renography is to no small extent responsible for the very divergent results reported in the literature Some authors question whether the renogram allows quantitative analysis of renal function (Spencer et al 1961 Pircher et al 1963)

Our technique has however given fairly reproducible results and the model experiments where the shielding

Figs 30 a and e that cases were seen with about 50 per cent impairment of function ([131]I Hypaque and creatinine clearance) with an uptake ratio that lay within the normal range This means that [131]I Hypaque does not satisfactorily differentiate between diseased and normal kidneys Other substances such as [131]I Hippuran with its *high clearance and high concentration* in the kidney which is reflected by *the slope of the second segment of the* renogram should provide a much better possibility for obtaining a measure with a narrower range There should be a much smaller risk of overlap between the pathological and normal curves and thereby the value of these external measurements is increased

A number of investigators have compared the renogram and different renal function tests Serratto et al (1959) reported that blood urea between 16—*20 mg/100 ml is not uncommon in* patients with a normal [131]I Diodrast renogram When the blood urea exceeded 20 mg/100 ml the incidence of bilaterally abnormal renograms was high *(27/32)* Normal [131]I Diodrast renograms were not uncommon in patients in whom the urea clearance was *markedly reduced Substantially* decreased urea clearance was however always associated with bilateral abnormal renograms Oeff et al (1963) found that the [131]I *Hippuran* renogram could be normal even when the PAH and inulin clearance were decreased They recommended that if one wanted *to examine slight impair*ment of renal function clearance tests should be used because the renogram does not reveal mild impairment Similar experience has been made with radioactive *Diodrast* and *Hippuran* renogram by Roth et al (1960) Strafton & Garcia (1960), Cobb (1961) Minami et al (1961) zum Winkel et al (1961 c) Scholz et al (1964) and zum Winkel (1964)

A supplementary parameter mainly to the uptake ratio is the retention calculated from the external chest curve The linear relation between retention and renal function data for [131]I Hypaque and creatinine clearance shows that retention could be used as a measure of renal function Retention together with the external renal measurements makes it possible to estimate total and individual renal function Such types of parameters have *been used by Winter (1961) and zum* Winkel (1964)

One of the most important purposes *of renography* is *to compare the two* sides and show any disparity between them In Figs 27 and 28 it was easy to compare not only the individual but also the pairs of values in the individual patients This form of diagrammatic illustration allows direct evaluation of the distance between the values on the sides compared and at the same time their localisation within the fields The procedure implies a considerable *simplification and facilitates comparisons between the different* series of diseases This evaluation with only two parameters calculated *from* the second and third *segments of the* curves can be used also for renograms *made with other* substances such as [131]I *Hippuran* One can extend the

renal curves distributed among different fields agrees with the localisation of the renograms from the clinical series This agreement means that parameters calculated from the theoretical renal curve can be applied to the more complex renogram and thus be suitable for clinical use

The fields obtained from the clinical series then offer a possibility to determine the individual ratios for each case in the series with and without renal disease It is clear from the discussion in Chapter VIII C where a number of evaluation techniques were described, that evaluation of the renograms by visual inspection alone is not satisfactory This technique implies above all a risk of subjective interpretation by the examiner It should be underlined, however, that one must consider the general shape of the curve and any changes possibly due to less satisfactory conditions during the examination (changes in patient's position because of pain, or nervousness etc)

Parameters calculated as relative values from the renogram proved the most suitable provided that their calculation was not too complicated and laborious for routine clinical use It is also of importance that the parameters can be calculated from all types of curves which is difficult in inter alia slope analysis with tangents or determination of t_{max} (Brown et al 1963, Krogsgaard & Faus 1964) As discussed in Chapter VIII C t_{max} has however, often been used as a parameter It is probable that this parameter, though dependent on both uptake and excretion function, may be valuable in comparison between the two kidneys of a given patient (Scholz et al 1961) t_{max} was found less suitable for the ^{131}I Hypaque renogram, but may be more informative in renograms made with substances with high clearance rate e g ^{131}I Hippuran (zum Winkel et al 1961 c, Stewart & Haynie 1962, Wax & McDonald 1962)

In order to find out what the renogram measures, the linear relation between the uptake ratio and renal function data obtained with ^{131}I Hypaque and creatinine clearance respectively maximal specific gravity, showed that the choice of a ratio calculated from the second segment was justified as a measure of renal function This ratio however, gives no numerical value for glomerular filtration or tubular secretion separately The same good relation between the parameters applied to the second segment and renal function data have been reported for ^{131}I Hippuran by Becker et al (1963) Wax & McDonald (1964) and zum Winkel (1964) while Taplin et al (1963) found a good correlation between renal blood flow (clearance of Radio Hippuran) and the slope of the second segment Block et al (1964) found a less good correlation between uptake T 1/2 of the second segment of the ^{131}I Hippuran renogram and PAH clearance while ^{131}I Diodrast had formerly been found to show a good correlation between this parameter and the 30 minute excretion of ^{131}I Diodrast (Block et al 1960 a)

As to the sensitivity of the ^{131}I Hypaque renography it is clear from

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procedure by giving the results as percentages of the normal values of the parameters and obtain a numerical relation between the kidneys

In the comparison between urograms and renograms the series with parenchymal renal disease, including the hypertension series, agreement was found in 21 of 33 cases On comparison of the renographic findings with the results of urography, laparotomy and post mortem examination the corresponding values were 33 of 44

The lack of agreement can be explained by the fact that urography and renography are not strictly comparable from a functional point of view The extensive use of excretion urography in clinical investigations of renal diseases, however justifies such a comparison If the renogram is abnormal in a patient without urographically demonstrable changes, it does not necessarily mean that the renogram is wrong, but simply that there is no close correlation between the two methods

Among cases with a discrepancy between the tests there were seven where the urograms were normal, but the renograms bilaterally abnormal without any difference between the sides In six of the cases, however the total function was decreased which corresponded to the renogram The urograms was thus less sensitive to impairment of renal function, which is in agreement with the results reported by Edling et al (1956) and Squire & Schlegel (1959), who compared the urographic findings with selective clearance determinations *e g* regarding PAH and inulin Similar results with normal urograms and bilateral abnormal renograms where the total function corresponded to the renograms have been reported by Serratto et al (1959), Straffon & Garcia (1960) Winter (1960) and zum Winkel (1964) In the other cases with a discrepancy between the tests, it could be explained by insignificant morphological changes in the urogram not having produced any impairment of renal function

In the series not examined with urography because of impaired renal function but examined post mortem good agreement was invariably found between the bilateral changes demonstrated by the renogram and the results of autopsy The renograms also showed agreement with results from renal function tests

In the eight cases where the renogram showed disparity between the kidneys the corresponding urogram revealed either morphological changes with poor density in the pelvis or significant differences in the size of the kidneys In this series it was remarkable that total renal function determined by creatinine clearance serum creatinine or N P N provided no guidance These values were normal in all the patients and thus give no information about the differences between the two kidneys in each patient Determination of total function by clearance tests is thus of limited value in deciding whether a patient has unilateral impairment of renal function and in such cases the renogram may prove a helpful guide to the clinician

in the further investigation of the case (roentgen examinations and selective clearance tests)

While comparison between urography and renography in the above mentioned series was complicated by the fact that urography has certain drawbacks as a function test, the relationship was better in the evaluation of different forms of urinary obstruction. Since its introduction in the 1930s excretion urography has proved the most reliable method for demonstrating urinary obstruction especially in acute situations but also in chronic urinary obstruction (Boeminghaus 1932 Hellmer 1935 Wulff 1936 and others). Good agreement was found between the clinical and roentgenological findings both in the acute stage and in the later follow up. As far as renography is concerned the most important question is whether the method and especially its parameters (excretion ratios (15/30 and 5/15 see page 67) are sensitive enough to demonstrate urinary obstruction in the acute stage as well as in the chronic stage of urinary obstruction of varying origin. The choice of excretion ratio 15/30 was therefore made in order to obtain as true a renographic measure as possible of the elimination of the test substance from the area of the kidney.

It is clear from Fig. 27 a that the values found for cases with urinary obstruction fell within the upper left field. The values for the contralateral healthy side and cases where obstruction could be excluded were mostly within the normal field. The difference in excretion ratio was sufficient to eliminate the roentgen verified cases of urinary obstruction.

While the difference in excretion ratio is most useful in deciding whether a patient has urinary obstruction or not the difference in uptake ratio is of importance because it also reveals any difference in function between the two kidneys. The patients in the acute obstruction series with difference in uptake ratio belonged to the group with pain during the test and therefore unable to sit still. This may explain the difference in uptake since the patients had no history of renal disease. In the present investigation urinary obstruction was the only disease that caused a typical change in the appearance of the renogram with a decreased excretion ratio provided that uptake function was normal. If urinary obstruction can be excluded, the renogram reflects only the uptake function. These results are in agreement with those reported by Burbank et al (1963) Farmelant et al (1964) Scholz et al (1964) and zum Winkel (1964).

It should however be underlined that this appearance of the renogram is also seen in other states such as a low rate of urine flow of varying cause (Wedeen et al 1963). This type of curve has been produced experimentally and seen clinically in severe dehydration and in a faint (Winter 1956 1963 O'Conor et al 1961 Wax & McDonald 1962). In the present material the patients had moderate hydration and were not intentionally dehydrated. Exceptions to this rule were

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cases with suspect urinary obstruction where the patients did not drink before the examination. This series thus included patients with pain, nausea and vomiting, without clinical signs of any severe dehydration or blood pressure fall resembling shock. The good agreement between the renograms and urograms did not provide any evidence for "false" positive obstruction curves due to extreme dehydration.

Renography showed that it was in chronic pyelonephritis, hypertension, other renal parenchymal disease and in polycystic disease that disparity was found between the two kidneys in the series with renal parenchymal disease. These results are in agreement with those reported by other investigators (Winter 1956, 1957, Winter et al. 1959, Block et al. 1960 a, zum Winkel et al. 1961 c, Dore et al. 1962, Stewart & Haynie 1962, Burbank et al. 1963, Schutterle & zum Winkel 1963, Farmelant et al. 1964, Scholz et al. 1964, zum Winkel 1964).

One of the most important points discussed in the literature is whether the renogram is of any value as a screening test for demonstrating unilateral renal injury in hypertension, where renal changes are due to stenosis of the renal arteries or parenchymal damage, such as unilateral chronic pyelonephritis. Since the first preliminary publications by Winter (1956, 1957) the literature in this field has become voluminous. Most of the publications are however confined to case reports, large series still being few.

Favourable results have been presented by several groups of workers who used radioactive Diodrast, Hypaque or Hippuran renograms alone or combined with urography with rapid film technique in the screening of the patients with unilateral vascular disease from other cases with hypertension (Winter et al. 1959, Block et al. 1960 a, Maxwell et al. 1960, Burrows et al. 1961, Tauxe 1961, Hunt et al. 1962 b, Maxwell 1962, Whitley et al. 1962, Burbank et al. 1963, Doig et al. 1963, Maxwell et al. 1964, Wall & Whalen 1965).

According to Stewart & Haynie (1962) the ^{131}I Hippuran renogram is ineffective as a screening test since it gives a high percentage of false positive (25 %) and negative (27 %) results. Similar experience has been reported by Scott & Quesada (1962) and Fris & Krogsgaard (1964). One possibility of reducing the frequency of false positive and negative results would be to use an evaluation technique according to the principles described above. In the material presented cases with different renal diseases have been included to exemplify the renograms in these diseases. The material is too small to allow any conclusions about the frequency of certain pathological patterns in the different groups of diseases.

In recent years the use of the renogram showing different forms of urinary obstruction has received increasing attention. In series with acute suspect urinary obstruction the renogram was able to distinguish patients with different abdominal conditions

from cases with urinary obstruction due to stone The renogram has thus proved to be a valuable supplement to other examination methods Parts of the present material have been described in preliminary studies and the results have shown that the renogram is a good supplement to excretion urography (Denneberg 1959/60) The renograms proved especially valuable in those cases where the urography could not give any definite information in cases of suspect urinary obstruction without verified stone or in so called roentgenologically silent kidney Other investigators have shown that patients with ureteric stone can be followed with advantage by serial renography (Boyd et al 1959 Serratto et al 1959 Lawrence et al 1963 zum Winkel 1964)

Of interest was case M J in the series with acute suspected urinary obstruction Since the patient had only one kidney urography was not done because of the risk of complications The renograms showed no signs of obstruction Follow up after the acute stage verified that it was not a question of urinary stone but of cystopyelitis A related problem is that urographic investigation is contraindicated by an increased N P N Excretion urography with the dose necessary for roentgen examination makes such an examination hazardous besides which the quality of the films is less satisfactory Recording of the renogram however is not attended by any risk and an increased N P N does not contraindicate the examination It has been shown experimentally and clinically that renography can distinguish between acute anuria and oliguria due to acute bilateral obstruction acute tubular necrosis and acute dehydration during the first 48 hours (O Conor et al 1961 Wax & McDonald 1962 Lawrence et al 1963 Ross et al 1963 Trinkle & Kiser 1964)

The present series of acute urinary obstruction as well as in the hydronephrosis series included examples of so called roentgenologically silent kidneys (non visualized kidneys) This term is not tantamount to non functioning kidney but is a roentgenological classification of kidneys without uptake and excretion of contrast medium at urography Clearance examinations can be difficult to perform because little or no urine is excreted In such cases the renogram can give information on the possible uptake capacity of the kidney While this roentgenological picture is not uncommon in acute urinary obstruction (Olsson & Jönsson 1962) Spjut & Nicolai (1961) found that very few non visualized kidneys were completely normal Obstruction of the urinary tract and acute or chronic pyelonephritis were the commonest causes

Irrespective of the cause of the silent kidney such a finding leads to an extensive urologic investigation with cystoscopy ureteric catheterisation retrograde pyelography and renal angiography If the renogram in such cases shows signs of obstruction it is a sign that the kidney can recover its function after elimination of the obstruction which has been shown experimentally in animals by Denneberg

et al. (1961 b) and zum Winkel (1964).

In cases with non visualized kidney in the acute urinary obstruction series the renogram showed continuously ascending curves and pathologically decreased excretion ratios as a sign of the urinary obstruction. On the other hand the two cases with silent kidneys in the hydronephrosis series (cases F. J. and E. H.), showed no such uptake segment with a continuously rising curve as a sign of urinary obstruction, but only a falling curve of the type seen in Fig. 26, *i. e.* a kidney without function, which was verified at angiography and autopsy. In the cases in the hydronephrosis series (cases K. O., D. D., V. P., J. W. and B. P.) the renogram proved valuable as a supplement to the morphological roentgen examination in deciding whether operation should be performed or not. It should be underlined that the renogram offered a possibility of evaluating both the diseased and the healthy side. The function of the healthy kidney is of significance in contemplated nephrectomy of the diseased kidney (Hauge et al. 1962, Lawrence et al. 1963, Gasser & Hawliczek 1964). The value of the renogram in post operative gynaecological complications of the urinary tract (silent ureteral injury and abscess or cancer infiltration) can be exemplified by cases S. A. and E. E. in the hydronephrosis series. The renogram supplemented urography by showing preserved capacity of uptake and evidence of the functioning parenchyma. Good experience with ^{131}I Hippuran renography in gynaecological diseases and in complications of the urinary tract have been reported by various authors (Gerbie et al. 1961, 1962, zum Winkel et al. 1961 a, b, Quinn et al. 1962, Dische et al. 1963, West & Nordyke 1963, Roddick et al. 1964, zum Winkel 1964).

It is apparent from the data given above that the evaluation procedure with two parameters calculated from the second and third segments of the renogram provides a simple method for estimating the function of the individual kidney in clinical practice. The clinical examples illustrate the value of renography in the investigation of various renal diseases and especially for demonstrating functional disparity between the kidneys in hypertension, chronic pyelonephritis, other renal parenchymal diseases, polycystic disease and in urinary obstruction. Since renography allows examination of the function of each kidney separately, it is a valuable supplement to conventional clearance tests which measure only the total renal function and which are therefore of less value in the investigation of unilateral renal disease. Renography is also a useful supplement in cases where excretion urography cannot give sufficient information.

CHAPTER IX

GENERAL SUMMARY

Hypaque (sodium diatrizoate) is widely used as a contrast medium for excretion urography. Labelled with ^{131}I it is also used for external renal measurements. The purpose of the present investigation was to elucidate the distribution and excretion of radioactive Hypaque and its use in radioisotope renography. The material consisted of 183 hospital patients with and without renal disease and 10 healthy students.

After a single injection in normals the plasma curve fell rapidly and after two hours more than 90 per cent of the radioactivity had left the blood. This rapid elimination from the blood corresponded to the rapid excretion of the substance in the urine. The extrarenal excretion of the substance is normally negligible as seen *inter alia* from the low biliary 24 hour excretion. The 2 hour distribution volume of ^{131}I Hypaque was on the average 20 per cent of body weight.

^{131}I Hypaque clearance was studied after simultaneous administration of inulin, PAH and radioactive Hypaque with addition of varying amounts of non-radioactive Hypaque with and without blocking of the tubules by Probenecid. The mean ^{131}I Hypaque clearance was 115 ml/min with an observed range of 80—153 ml/min in the controls. The clearance values were not changed when the initial plasma level of Hypaque was varied between 0.002—6 mg/100 ml plasma.

It is known that Hypaque binding to serum proteins is negligible in man which means that the substance is completely filtrable *via* the glomerulus. The question whether also tubular secretion occurs was studied by comparing the clearances of ^{131}I Hypaque and inulin. The mean quotient between the clearances of the two substances was greater than 1.0, suggesting tubular secretion of ^{131}I Hypaque. Observations also argued for an almost linear relation between the Hypaque/inulin quotient and inulin clearance which at high inulin clearance gave a quotient of 1.0 or less. After administration of Probenecid the Hypaque/inulin quotient decreased, indicating blocking of the secretion of ^{131}I Hypaque. On the basis of these results it was concluded that ^{131}I Hypaque is eliminated largely by glomerular filtration but also to some extent by tubular secretion; certain observations also argue for the occurrence of tubular reabsorption. The two last mentioned processes compensate one another

more or less completely. This means that even though ^{131}I-Hypaque is not a true measure of glomerular filtration, it should be clinically useful as a substitute for inulin clearance.

To find out the most suitable technique for measurement of single injection clearance three types of such clearances (total, 2- and 24 hour renal clearances) were compared with intravenous infusion clearance. This comparison showed a good correlation with the 2- and 24 hour clearances, while the values obtained for total clearance were systematically too high because the plasma curve for ^{131}I-Hypaque had not reached its final slope at 2 hours. The good agreement suggests that the 2- and 24 hour clearances could replace the more complicated clearance with the infusion technique, besides which it can be done simultaneously with renography.

A new method of evaluation of the external renal curves (renograms) is presented. The processes in the renal handling of the test substance were summarized as inflow and outflow of the substance from the region of the kidney. In order to obtain a quantitative measure of these two processes ratios were calculated from the rising (uptake ratio) and falling (excretion ratio) limbs of the curve. These parameters may be either normal or decreased and four possible combinations are thus obtained which represent four different types of functional disturbances. The four types fall into different fields when the two parameters are plotted against each other in a diagram. The appearance of the renogram is also affected by extra renal factors (the radioactivity in the blood, extra vascular space and bladder). Even when the two ratios were calculated for patients with a known type of renal functional disturbance, the position of the parameters in the diagram were in agreement with the above theoretical analysis.

That the uptake ratio is a good measure of parenchymal function of the kidney is apparent from the good correlation found between this parameter and renal function data obtained with radioactive Hypaque (clearance with infusion technique, 24 hour urinary recovery, disappearance from the blood) and other tests on renal function (creatinine clearance, serum creatinine, N.P.N. and maximal specific gravity).

That the excretion ratio reflects changes in the elimination from the kidney was shown by the fact that this ratio could distinguish cases of urinary obstruction (acute or chronic) from unobstructed cases. The parameters selected and the diagrammatic presentation of their values therefore appear to be useful in the evaluation of the renogram in clinical practice.

One of the most important purposes of renography is to compare the two sides and show any disparity between them. The proposed diagram allows easy evaluation and comparison not only of the individual but also of the pairs of values in a given patient. This procedure also facilitates comparison between the different types of renal diseases. This evaluation with only

two parameters calculated from the second and third segments of the curves can be used also for renograms made with other labelled substances

Renographic disparity between the two kidneys was demonstrated by differences between the uptake or excretion ratio on either side Such disparity was found in hypertension chronic pyelonephritis other renal parenchymal diseases and in polycystic disease

A comparison between the renographic findings and findings at urography laparotomy and autopsy showed agreement in two thirds of the cases with parenchymal renal disease including hypertension The lack of agreement in the remaining third could partly be explained by the fact that urography and renography are not strictly comparable from a functional point of view as was shown in cases with decreased clearance values having bilaterally pathological renograms but normal urograms

Cases with renographic disparity had urographic signs of morphologic changes with poor density in the pelvis or significant differences in the size of the kidneys Total renal function determined by clearance tests was normal and yielded no information about the differences between the two kidneys in a given patient Determination of total renal function is thus of limited value in deciding whether a patient has unilateral impairment of renal function and in such cases the renogram is useful in the further investigation

In the remaining cases with discrepancy between the tests this could be explained by small morphological changes in the urogram not having produced any impairment of renal function In the series not examined with urography *because of impaired* renal function but examined post mortem good agreement was invariably found between the bilateral changes demonstrated by the renograms and the results of autopsy and renal function tests

In the series with suspected acute urinary obstruction the renogram was able to the distinguish patients with various abdominal conditions from patients with urinary obstruction due to stone Renography thus proved to be a valuable supplement to other renal examinations Renography also proved valuable in those cases where excretion urography could not give any definite information in cases of suspect urinary obstruction without verified stone or in roentgenologically silent kidneys It also proved useful to estimate renal function on both sides before contemplated nephrectomy for hydronephrosis and at follow up of renal function after gynaecological operations with complications from the urinary tract

Acknowledgements

Thanks go above all to my chief Jan Waldenström M D Professor of Internal Diseases of the University of Lund for valuable suggestions and stimulation throughout the entire investigation

The investigation was started under the supervision of the late Docent Bengt Skanse who offered most gener

ous help, for which I feel deeply indebted

To Docent Bertil Nosslin I which to express my thanks for his valuable advice and constructive criticism

Thanks for help in various respects are due to Professor Folke Linell, Ingenjor Inge Hedenskog, Docent Berndt Waldeskog and Doctor Lennart Wehlin

The investigation was supported by grants from the Medical Faculty of the University of Lund and Ernhold Lundstroms stiftelse

DETERMINATION OF CLEARANCE AND DISTRIBUTION VOLUME WITH THE SINGLE INJECTION TECHNIQUE

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Theoretical analysis of the kinetics of a substance deposited in an open biological system by single injection has been the subject of numerous papers since Teorell s (1937 a and b) publication of the fundamental principles Reviews of compartment analysis have been given by Dost (1953) Sheppard (1962) and Riggs (1963) The theoretical treatment of the clearance problems dealt with here is based on these conventional principles but is at the same time focused especially on those problems encountered in the analysis of data obtained in experiments with test substances eliminated entirely or partly by the kidneys

Symbols

- t = time (min)
- P = total amount in plasma (fraction of dose)
- V = plasma volume (ml)
- D = distribution volume (ml)
- Q = total amount excreted from plasma (fraction of dose)
- U = total amount excreted by renal clearance (fraction of dose)
- C = total clearance from plasma (ml/min)
- C_R = renal clearance from plasma (ml/min)
- F = urinary flow (ml/min)
- S = renal tract dead space (ml)
- k_P = fractional rate of total plasma clearance = C/V (min $^{-1}$)
- k_R = fractional rate of renal plasma clearance = C_R/V (min $^{-1}$)
- A = total area under the P curve (min)
- A_t = area under the P curve to time t (min)
- c = coefficient of exponential term describing the P curve (fraction of dose)
- b = exponential constant in the expression for the P curve (min $^{-1}$)

Assuming that the injected substance mixes immediately with the plasma the initial theoretical concentration of the substance will be the value obtained on division of the dose by V This value is taken as 1 and all later values are expressed with reference to this value and thereby show what proportion of the dose is circulating in the plasma at the time of sampling

It is also assumed that the plasma curve can be described by an arbitrary number of exponential functions

$$P = c_1e^{-b_1t} + c_2e^{-b_2t} + \quad = \sum ce^{-bt}$$

The total area under the P curve is obtained by integration of this expression between t = 0 and t = ∞, which gives

$$A = \frac{c_1}{b_1} + \frac{c_2}{b_2} + \quad = \sum\frac{c}{b}$$

The area under the P curve for a definite time (A_t) can be calculated either as the difference between A and the remaining area after time t (calculated in analogous manner), or by direct measurement by some conventional method (planimetry, weighing, counting of squares, numerical calculation with integration formulae etc)

I Total plasma clearance

Since the total excretion must equal total clearance we get

$$dQ/dt = K_P P$$

Integration gives

$$Q = K_P \int P\,dt$$

After an infinite period the entire dose is excreted which gives

$$1 = K_P \int_0^\infty P dt = K_P A$$

Since K_P according to definition is $= C/V$ we finally get

$$C = \frac{V}{A} \tag{1}$$

The total clearance is thus calculated as the quotient between the plasma volume and the total area under the plasma curve This formula holds for all substances added *to the plasma* (whether as a single injection or by infusion) and is independent of the co occurring distribution between the plasma and the extravascular space The only requirement is that clearance is constant during the observation period When P is expressed as a fraction of the dose, the area A has the dimension time It can be shown that this time is the mean time the substance remains in the plasma It corresponds to the turnover time in kinetic analysis of metabolic processes

II Renal clearance (uncorrected for dead space)

This clearance is calculated analogously to that for total clearance

$$dU/dt = K_R P$$

Integration gives

$$U = K_R \int P\,dt$$

At time t

$$U_t = K_R \int_0^t P\,dt = K_R A_t$$

Since $K_R = C_R/V$ we get

$$C_R = \frac{U_t V}{A_t} \tag{2}$$

However in practice this formula gives systematic errors since U_t refers to all substance that has been excreted by the kidney i e the sum of the amount in the renal tract plus the amount in the bladder Since only the amount that has reached the bladder

can be measured the value observed is too small and C_R correspondingly too low

III Correction for renal tract dead space delay

This dead space has two effects, both causing a delay in the excretion measured namely a transport delay because of the time the substance needs to run through the urinary tract and secondly a mixing delay because during its passage newformed urine is mixed in the dead space with previously secreted urine An adequate correction should cover both types of delay (Bojesen 1954) With the aid of the known urine flow a simple approximative calculation of this correction can be done as follows

a) Transport delay As pointed out by McSwiney & DeWardener (1950) this may be conceived as the quotient between the dead space volume and the urine flow *i e* delay time is S/F
b) Mixing delay This can also be regarded as a function of dead space volume and urine flow Bojesen (1954) dicussed this problem in detail and showed that with a linearly falling plasma curve the mixing delay time is S/F for an exponentially falling curve the corresponding expression is S/F (1 + bS/2I) When the curve falls slowly the difference between the two expressions is negligible

The total delay correction may thus be taken as 2 × S/I Generally only I is known so that S must be estimated. McSwiney and DeWardener (1950) determined the minimum transport time at different urine flows and showed that the calculated dead space varied regularly with the magnitude of the flow which is also apparent from their Fig 1 Bojesen (1954), who used dogs showed that, except when the urine flow is very high or very low the dead space volume increases linearly with the flow

The following regression formula was calculated from data given by McSwiney and DeWardener (3 cases with lowest rate of urine flow excluded)

$$S = 3\ 28 + 1\ 82\ F$$

This gives the following expression for total delay correction

$$T = 2\ S/F = 3\ 6 + 6\ 6/F \qquad (3)$$

This formula holds when the urine is collected under the conditions used by McSwiney & DeWardener (1950), *i e* continuous collection via catheter The dead space of the catheter (2 ml) is thus included in the calculated dead space volume The following values for total correction for different urine flows are obtained

Flow (ml min)	Correction (min)	Flow (ml,min)	Correction (min)
1 0	10 2	5 0	4 9
1.5	8 0	6 0	4 7
2.0	6 9	7 0	4.5
2.5	6.2	8.0	4 4
3.0	5.8	9 0	4.3
4 0	5.3	10 0	4.3

When the bladder urine is collected intermittently (spontaneous voiding or by catheter with rinsing) the volume

It is also assumed that the *plasma* curve can be described by an arbitrary number of exponential functions

$$P = c_1e^{-b_1t} + c_2e^{-b_2t} + \quad = \sum ce^{-bt}$$

The total area under the P curve is *obtained* by integration of this expression between t = 0 and t = ∞, which gives

$$A = \frac{c_1}{b_1} + \frac{c_2}{b_2} + \quad = \sum \frac{c}{b}$$

The area under the P curve *for* a definite time (A_t) can be calculated either as the difference between A and the remaining area after time t (calculated in analogous manner), or by direct measurement by some conventional method (planimetry, weighing, counting of squares, numerical calculation with integration formulae etc)

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$$U_t = K_R \int_0^t P\,dt = K_R A_t$$

Since $K_R = C_R/V$ we get

$$C_R = \frac{U_t V}{A_t} \qquad (2)$$

However in practice this formula gives systematic errors since U_t refers to all substance that has been excreted by the kidney *i e* the sum of the amount in the renal tract plus the amount in the bladder Since only the amount that has reached the bladder

arterial value The higher the clearance rate the more pronounced this difference (Brun et al 1949) Since renal clearance occurs from arterial blood arterial or capillary blood should be used for determination of the plasma level If venous blood is used the value obtained for the area A might be too small which according to the formula (1)—(3) results in too high a value for clearance However the longer the venous curve is followed the smaller this error, since negative and positive differences tend to compensate one another There is theoretical evidence that this compensation is complete if the venous curve is calculated to infinity It should thus be possible to obtain a correct value for the total clearance from measurement of venous blood according to the formula (1) and a correct value for renal clearance according to formula (3) if the urine is collected for a long time *e g 1 day*

In tests with some substances the plasma curve may require a long time to *reach its true final slope* If sampling is ceased before it has reached its final slope and the then final part of the curve is extrapolated to infinity the area under the curve will be too small and the total clearance value too large At the same time the calculated *total distribution volume will* be too small The shorter time the plasma curve is followed the greater the error It is thus better to sample at relatively long intervals for a long time instead of at short intervals for a short time Calculation of renal clearance according to formula (3) avoids this source of error because with this formula one can use a directly measured limited part of the total area under the plasma curve This possible source of error of the total clearance should be borne in mind in the calculation of extrarenal clearance as the difference between total and renal clearance

References

Andersen S B Metabolism of Human Gamma Globulin (γ_{ss} globulin) Blackwell Scient. Publ Oxford 1964

Bojesen E. Acta physiol *scand 32 39 1954*

Brun C. Hilden T & Raaschou F J clin. Invest *28* 144 1949

Dost F H Der Blutspiegel Thieme Leipzig 1953

McSwiney R R & DeWardener H E. Lancet *II* 845 1950

Riggs D S The Mathematical Approach to Physiological Problems Williams & Wilkins Co Baltimore 1963

Sheppard C. W Basic Principles of the Tracer Method J *Wiley & Sons Inc* New York 1962

Teorell T Arch internat. Pharmacodynam *57* 205 1937 a

— *Arch internat.* Pharmacodynam. 57 226 *1937 b*

of the catheter is not included with the dead space and the expression for dead space should be reduced by 2 ml

$$S = 1\,28 + 1\,82\,F$$

$$T = 3\,6 + 2\,6/F \qquad (4)$$

The total correction will then be

Flow (ml/min)	Correction (min)	Flow (ml/min)	Correction (min)
1 0	6 2	5 0	4 1
1 5	5 3	6 0	4 0
2 0	4 9	7 0	4 0
2 5	4 6	8 0	3 9
3 0	4 5	9 0	3 9
4 0	4 3	10 0	3 9

The correction, as expected, is greatest at low flow and at high flow it asymptotically approaches the limit of 3 6 min The larger the flow rate the smaller effect of the dead space of the catheter At a urinary secretion rate of about 5—10 ml/min the correction is about 4 min which is some what more than the 2—3 min which have so far often been used and which correspond to "first appearance time" *i e* transport delay

The formulae for delay correction show the importance of a high urine flow in clearance studies Moreover, in tests using the single injection tech nique the relative importance of cor rection decreases with increasing dura tion of the collection period used High urine flow and a long collection period are therefore desirable

IV Renal clearance (with correction for dead space)

The time correction calculated in the previous section should by rights be applied *in the form of a prolongation* of the time for the collection of urine If the plasma disappearance curve and the curve for the excretion rate are *parallel, correction may equally well* be done by a corresponding subtrac tion of time in reading the plasma value This is in practice simpler, since *the plasma curve is followed* con tinually If the urine is collected from the beginning of the test to the time t, the area under the plasma curve should thus be measured to time t T The formula for renal clearance is then

$$C_R = \frac{U_t V}{A_{t\,T}} \qquad (5)$$

V Distribution volume

If the plasma curve is followed until it reaches its final slope and is resolved into exponential terms, the total dis tribution volume can be calculated ac cording to the following formula

$$D = V\frac{\Sigma\,(b/c^2)}{(\Sigma\,b/c)^2} \qquad (6)$$

This formula holds for all sub *stances that are not eliminated directly* from the extravascular space The derivation of the formula is given in Andersen (1964 Appendix G)

VI Comments

In all tests with the single injection technique there is an arteriovenous difference in the plasma values In the beginning the venous value is lower than the arterial value it becomes momentarily identical with and then remains constantly higher than, the

APPENDIX

(Tables IX—XX)

Table IX Exponential constants (c) and intercepts (b) found by slope analysis in 31 cases used in study of clearance of Hypaque with single injection technique (Chapter VII)

Case	Intercepts				Exponential constants			
	c_1	c_2	c_3	c_4	b_1	b_2	b_3	b_4
LN	0 162	0 132	0 211	0 495	0 00737	0 0667	0 230	1.330
HI	143	157	309	391	772	426	344	1 262
SP	159	142	255	444	686	529	356	1 228
KA	172	196	225	407	937	672	297	0 849
DA	144	157	353	346	711	545	356	0 796
WJ	167	227	606		866	698	495	
LL	135	133	255	477	745	458	245	0 939
HB	171	176	206	447	704	894	320	1 146
AJ	143	165	265	427	987	635	279	1 108
EK	206	181	613		1005	658	313	
SN	161	134	314	391	845	579	191	0 831
SA	204	299	497		963	680	431	
HM	163	163	294	380	783	509	307	1 064
MI	157	157	323	363	892	590	237	0 887
AO	225	191	274	310	726	529	301	1 425
RA	127	096	038	679	630	529	181	1 044
BE	159	112	193	536	927	536	177	0 939
BA	167	159	230	444	737	606	354	2 494
GA	172	076	279	473	779	512	186	1 064
HJ	225	284	491		553	931	939	
HG	176	134	216	474	663	480	242	1 451
AS	183	274	353	190	758	980	356	2 763
WA	174	122	245	459	529	485	237	0 798
LB	167	142	274	417	571	447	270	1 413
AA	173	152	057	618	419	456	135	0 458
IG	202	186	167	445	508	837	419	1 257
KK	206	046	142	606	360	408	154	0 783
AO	197	304	270	229	258	954	307	0 939
LV	230	113	216	441	219	614	230	1 124
TB	288	157	304	251	250	470	237	0 854
BI	257	056	221	436	184	731	341	0 979

Table X Urine recovery, clearance of Hypaque with single injection and intravenous infusion technique and distribution volume in 31 cases (Chapter VII)

Case	Urine flow	Urine recovery		Renal clear [1]		Total[1] clear	Infusion[1] clear	Distr volume
		2 h	24 h	2 h	24 h			
	ml/min	%	%	ml/min	ml/min	ml/min	ml/min	ml min
EN	2 1	51	95	120	141	149	129	*17800*
IH	1 9	62	96	120	125	130	124	13500
SP	*3 6*	*54*	95	112	122	128	124	16100
KA						161		14300
DA						154		18300
WJ						146		14200
LL						108		12000
HB						*86*		*11000*
AJ			98		162	165		13500
EK			96		132	138		11400
SN			91		115	126		12400
SÅ			95		96	101		8600
HM			95		95	100		10700
ML	0 4	62	93	147	147	158	131	14600
AO	3 0	60	102	99	102	102	116	12400
RA	2 8	48	95	99	114	120	108	16800
BE	1 8	60	93	110	119	128	107	11600
BA	0 4	50	91	105	108	119	107	14300
GÅ						124	87	14000
HJ	*1 9*	*43*	*86*	*81*	*82*	*96*	*86*	*15900*
HG	2 7	56	97	77	79	81	78	10700
YS	3 3	52	100	88	108	108	77	12500
WA			93		62	67	73	11300
EB	3 6	53	100	74	75	75	60	11600
AA	1 5	34	89	61	71	80	60	17000
IG	2 1	35	80	45	48	60	49	11100
KK	*1 4*	*31*	*98*	*35*	39	*40*	36	10600
AÖ	1 5	9	63	15	32	51	23	18600
LV	2 9	11	49	15	17	35	15	15200
TB			49		15	31	15	12100
BF	0 8	2	17	3	5	27	4	*14300*

1 The clearance values are not corrected to hold for 1 73 m^2 body surface

Table XI Series A 1 (Chapter VII) Clearance of Hypaque inulin and PAH with intravenous infusion technique quotient Hyp/inulin clearance and filtration fraction.

Case	Hcr	Period		Urine flow	Clearance			Quotient Hyp Inulin	Filtration Fraction ×100
		No	duration		Hyp	Inulin	PAH		
	%		min	ml min	ml min	ml min	ml min		
MA	39	1	20	4 1	122	79	303	1 54	26
		2	20	2 9	118	97	376	1 22	26
		3	20	3 0	130	131	647	0 99	20
		M[1]			123	102	442	1 21	23
		M corr[2]			109	91	393		
UA	31	1	20	2 2	89	116	711	0 77	16
		2	20	1 1	100	87	466	1 15	19
		3	31	1 0	100	105	542	0 95	19
		M			96	103	573	0 94	18
		M corr			*103*	*110*	*613*		
EO	30	1	33	3 3	73	53	232	1 38	23
		2	26	1 1	91	109	389	0 83	28
		3	26	1 4	89	89	392	1 00	23
		M			84	84	338	1 01	25
		M corr			99	99	399		
DS	37	1	25	1 0	118	100	499	1 18	20
		2	25	1 1	151	81	537	1 86	15
		3	25	0 8	145	102	483	1 42	21
		M			138	94	506	1 46	19
		M corr			*106*	*73*	*388*		
DJ	39	1	20	1 5	135	116	669	1 16	17
		2	20	1 5	134	126	763	1 06	17
		3	20	1 6	135	117	672	1 15	17
		M			135	120	701	1 13	17
		M corr			136	121	708		
JL	42	1	20	1 4	150	130	691	1 15	19
		2	20	3 3	167	131	820	1 27	16
		3	20	7 9	152	144	744	1 06	19
		M			156	135	752	1 16	18
		M corr			136	118	656		
M	40	1	20	6 8	114	123	660	0 93	19
		2	20	6 3	117	76	381	1 54	20
		3	20	4 9	118	115	604	1 03	19
		M			116	105	548	1 11	19
		M corr			119	108	564		

Case	Hcr	Period		Urine flow	Clearance			Quotient Hyp / Inulin	Filtration Fraction ×100
		No	duration		Hyp	Inulin	PAH		
	%		min	ml/min	ml/min	ml/min	ml/min		
SH	39	1	20	12 9	123		587		
		2	20	10 5	117		571		
		3	20	8 7	118		524		
		M			119		561		
		M corr			108		511		
AB	41	1	20	2 7	112	110	589	1 02	19
		2	20	2 8	104	76	541	1 37	14
		3	20	3 9	139	137	637	1 01	22
		M			118	108	589	1 10	18
		M corr			105	96	524		
SP	38	1	20	1 6	136	136	594	1 00	23
		2	20	1 6	120	151	704	0 79	21
		3	20	2 1	115	140	629	0 82	22
		M			124	142	642	0 87	22
		M corr			114	130	591		
IH	39	1	20	9 0	140		457		
		2	19	10 6	114		374		
		3	19	11 1	117		415		
		M			124		415		
		M corr			126		423		
SL	35	1	20	4 7	82		505		
		2	20	3 3	95		408		
		3	20	5 6	88		503		
		M			88		472		
		M corr			80		430		
NS	41	1	25	11 0	120	69	408	1 74	17
		2	25	1 4	159	97	651	1 64	15
		3	20	2 1	145	134	614	1 08	22
		M			141	100	558	1 41	18
		M corr			117	83	464		
GZ	41	1	20	14 6	133	112	701	1 19	16
		2	20	14 3	138	100	687	1 38	15
		3	20	8 6	132	107	678	1 23	16
		M			134	106	689	1 26	15
		M corr			106	84	544		

Case	Hct	Period		Urine flow	Clearance			Quotient Hyp / Inulin	Filtration Fraction
		No	duration		Hyp	Inulin	PAH		
	%		min	ml/min	ml/min	ml/min	ml/min		×100
EN	38	1	20	7 6	131	122		1 07	
		2	21	8 1	130	138		0 94	
		3	20	6 4	125	139		0 90	
		M			129	133		0 97	
		M corr			111	114			
BK	42	1	20	3 2	109	80	311	1 36	26
		2	20	5 8	101	87	457	1 16	19
		3	20	8 2	103	54	220	1 91	25
		M			104	74	329	1 42	22
		M corr			100	71	316		
NK	43	1	20	11 0	122	106		1 15	
		2	20	10 0	117	134		0 87	
		3	20	4 9	101	161		0 63	
		M			113	134		0 85	
		M corr			98	117			
JJ	38	1	25	1 5	154	115	494	1.34	23
		2	25	2 5	139	125	505	1 11	25
		3	25	5 5	136	96	361	1 42	27
		M			143	112	454	1 28	25
		M corr			142	111	449		

[1] Mean value of 3 clearance periods
[2] Mean value corrected to hold for 1 73 m^2 body surface

Table XII Series A 2, 3, 4 (Chapter VII) Clearance of Hypaque with intravenous infusion technique

Case	Period		Urine flow	Hypaque clearance
	No	duration min	ml/min	ml/min
GJ	1	20	1 1	153
	2	21	1 1	142
	3	20	1 0	136
	M[1]			144
	M corr			147
RE	1	21	0 6	84
	2	29	0 6	104
	3	21	1 0	104
	M			97
	M corr			114
MN	1	22	1 9	75
	2	22	4 6	88
	3	22	4 6	100
	M			91
	M corr			91
US	1	23	0 4	106
	2	26	0 4	93
	3	21	0 5	118
	M			110
	M corr			142
VH	1	22	1 2	96
	2	22	0 8	84
	3	22	0 9	89
	M			90
	M corr			96
AD	1	23	0 9	129
	2	23	0 9	126
	3	29	1 6	137
	M			131
	M corr			117
FS	1	22	0 7	96
	2	22	0 7	115
	3	22	0 5	112
	M			108
	M corr			107

Case	Period No	Period duration min	Urine flow ml/min	Hypaque clearance ml min
MP	1	22	0 7	141
	2	22	0 8	133
	3	22	1 0	151
	M			142
	M corr			153
AP	1	22	0 7	98
	2	23	0 8	134
	3	22	0 6	116
	M			116
	M corr			125
GS	1	22	0 8	110
	2	22	0 7	133
	3	22	0 7	117
	M			120
	M corr			114
CJ	1	24	0 9	132
	2	25	0 4	90
	3	22	1 1	131
	M			110
	M corr			100

[1] Mean value of 3 clearance periods
[2] Mean value corrected to hold for 1 73 m^2 body surface

Table XII Series A 2, 3, 4 (Chapter VII) Clearance of Hypaque with intravenous infusion technique

Case	Period No	Period duration min	Urine flow ml/min	Hypaque clearance ml/min
GJ	1	20	1 1	153
	2	21	1 1	142
	3	20	1 0	136
	M[1]			144
	M corr[2]			147
RE	1	21	0 6	84
	2	29	0 6	104
	3	21	1 0	104
	M			97
	M corr			114
MN	1	22	1 9	75
	2	22	4 6	88
	3	22	4 6	100
	M			91
	M corr			91
LS	1	23	0 4	106
	2	26	0 4	93
	3	21	0 5	118
	M			110
	M corr			142
VH	1	22	1 2	96
	2	22	0 8	84
	3	22	0 9	89
	M			90
	M corr			96
AD	1	23	0 9	129
	2	23	0 9	126
	3	29	1 6	137
	M			131
	M corr			117
FS	1	22	0 7	96
	2	22	0 7	115
	3	22	0 5	112
	M			108
	M corr			107

Case	Hcr	Period		Urine flow	Clearance			Quotient Hyp / inulin	Filtration Fraction ×100
		No	duration min	ml/min	Hyp ml/min	Inulin ml/min	PAH ml/min		
RA	34	1	20	2.5	113	102		1 11	
		2	20	2 4	105	9		1 33	
		3	20	3 2	107	96		1 11	
		M			108	92		1 17	
		M corr			93	9			
FN		1	21	2 9	124	110	967	1 13	11
		2	21	3 6					
		3	21	1 3	88	62	496	1 42	13
		M			1 6	86	32	1 23	12
		M corr			30	3	620		
WA	37	1	20	1 9	[illegible]	60	407	1 1	15
		2	2	2	80	8	5 1	1 38	10
		3	20	2 0	6	53	518	1 43	1
		M			5	57	459	1.32	11
		M corr			88	[illegible]	84		
CA	41	1	24	5 9	86	51	268	1 69	19
		2	21	1 8	83	51	262	1 63	19
		3	21	1 2	92	63	26	1 46	21
		M			8	55	266	1 58	21
		M corr			8	4	2 1		
HC	[illegible]	1	20	5 1	5	56	2[illegible]	1 41	[illegible]
		2	21	2			315	1.32	18
		3	2	2.2	5	62	462	1 2	13
		M			8	8	351	1.33	1
		M corr			82	61	319		
VS	44	1	21	1 7	1	48	233	1 48	21
		2	2	2 3	88	96	281	0 92	34
		3	21	2 3	1	82	213	0 87	38
		M				5	242	1 12	31
		M corr			1	69	233		
BH	33	1	0	1	62	39	245	1.59	16
		2	24	7	68	35	220	1 94	16
		3	0	4 8	63	35	192	1 81	18
		M			64	36	221	1	16
		M corr			6	38	232		

Table XIII Series B (Chapter VII) Clearance of Hypaque inulin and PAH with intravenous infusion technique quotient Hyp/inulin clearance and filtration fraction

Case	Hcr	Period		Urine flow	Clearance			Quotient Hyp / inulin	Filtration fraction
		No	duration		Hyp	Inulin	PAH		
	%		min	ml/min	ml/min	ml/min	ml/min		×100
ÖD	39	1	20	10 1	129	90	678	1 43	13
		2	20	8 8	131	61	496	2 15	12
		3	20	4 4	118	102	763	1 16	13
		M[1]			126	84	646	1 49	13
		M corr[2]			117	78	601		
BE	33	1	20	1 1	102	81		1 26	
		2	20	2 0	103	81		1 27	
		3	20	3 7	115	86		1 34	
		M			107	83		1 29	
		M corr			116	90			
IE	41	1	22	1 8	120	65	327	1 85	20
		2	22	1 8	100	65	312	1 55	21
		3	22	2 5	131	88	585	1 49	15
		M			117	73	408	1 61	18
		M corr			111	69	388		
ML	42	1	20	2 5	131	119	523	1 10	23
		2	20	3 4	131	113	579	1 16	20
		3	20	4 2	132	172	733	0 77	23
		M			131	135	612	0 98	22
		M corr			110	113	514		
AO	34	1	23	1 8	130	101		1 29	
		2	22	2 3	124	90		1 38	
		3	25	1 4	95	109		0 87	
		M			116	100		1 16	
		M corr			107	92			
BA		1	27	1 4	121	90	376	1 34	24
		2	26	2 4	103	82	348	1 26	24
		3	22	2 8	97	89	274	1 09	32
		M			107	87	333	1 23	26
		M corr			96	78	300		
JA		1	20	4 9	86	38	244	2 28	16
		2	20	5 3	93	64	378	1 45	17
		3	22	4 7	102	68	417	1 50	16
		M			94	57	346	1 65	16
		M corr			96	58	353		

Case	Hct	Period		Urine flow	Clearance			Quotient Hyp / Inulin	Filtration Fraction ×100
		No	duration min		Hyp	Inulin	PAH		
	%			ml/min	ml/min	ml/min	ml/min		
BA	54	1	20	2.5	113	102		1.11	
		2	20	2.4	105	79		1.33	
		3	20	3.2	107	96		1.11	
		M			108	92		1.17	
		M corr			93	79			
CN		1	21	2.9	121	110	967	1.13	11
		2	21	3.6					
		3	21	1.3	88	62	496	1.42	13
		M			106	86	732	1.23	12
		M corr			90	73	620		
WA	37	1	20	1.9	70	60	407	1.17	15
		2	20	2.0	80	58	571	1.38	10
		3	20	2.0	76	53	518	1.43	10
		M			75	57	499	1.32	11
		M corr			88	67	584		
CA	41	1	24	5.5	86	51	268	1.69	19
		2	21	1.8	83	51	262	1.63	19
		3	21	1.2	92	63	267	1.46	24
		M			87	55	266	1.58	21
		M corr			85	54	261		
HC	35	1	20	5.1	79	56	277	1.41	20
		2	20	2	75	57	315	1.32	18
		3	20	2.2	79	62	462	1.27	13
		M			78	58	351	1.33	17
		M corr			82	61	309		
YS	44	1	21	1.7	71	48	233	1.48	21
		2	20	2.3	88	96	281	0.92	34
		3	21	2.3	71	82	213	0.87	38
		M			77	75	242	1.02	31
		M corr			71	69	233		
RH	[illegible]	1	20	7.1	62	35	249	1.59	16
		2	24	7.7	68	35	250	1.91	16
		3	20	4.8	63	35	192	1.80	18
		M			64	36	220	1.77	16
		M corr			67	38	232		

Case	Hcr	Period		Urine flow	Clearance			Quotient Hyp / inulin	Filtration Fraction ×100
		No	duration		Hyp	Inulin	PAH		
	%		min	ml/min	ml/min	ml/min	ml/min		
HJ	37	1	21	6.2	81	79		1.03	
		2	21	6.5	90	93		0.97	
		3	21	4.3	86	104		0.83	
		M			86	92		0.93	
		M corr			65	69			
FB	39	1	20	4.3	58	48	135	1.21	36
		2	20	2.6	59	48	124	1.23	39
		3	20	2.2	63	52	130	1.21	40
		M			60	49	130	1.22	38
		M corr			59	48	127		
AA	30	1	20	1.3	49	54	196	0.91	25
		2	20	1.9	58	55	169	1.00	34
		3	20	2.7	74	67	337	1.10	20
		M			60	60	234	1.01	26
		M corr			56	56	218		
IG	35	1	25	3.2	50	60		0.83	
		2	28	2.7	50	54		0.94	
		3	27	2.7	47	50		0.94	
		M			49	54		0.90	
		M corr			50	55			
KK	41	1	20	3.0	39	34		1.15	
		2	20	1.7	31	30		1.03	
		3	20	1.4	39	36		1.08	
		M			36	33		1.09	
		M corr			39	36			
AO	27	1	25	1.0	21	12	32	1.75	38
		2	25	1.1	27	18	43	1.50	42
		3	27	0.8	21	13	26	1.62	50
		M			23	14	34	1.60	44
		M corr			22	13	32		
IB	23	1	22	1.7	18	22		0.82	
		2	25	1.9	14	16		0.88	
		3	24	1.7	14	16		0.88	
		M			15	18		0.85	
		M corr			16	19			
LV	26	1	20	3.4	15	23		0.65	
		2	20	2.8	15	21		0.71	
		3	20	3.4	16	25		0.64	
		M			15	23		0.67	
		M corr			15	23			

[1] Mean value of 3 clearance periods
Mean value corrected to hold for 1.73 m^2 body surface

Table XIV Series C (Chapter VII) Clearance of Hypaque and inulin with intravenous infusion technique and quotient Hyp/inulin clearance before and after injection of Probenecid

Case	Probenecid	Period		Urine flow	Clearance		Quotient Hyp/Inulin
		No	duration		Hyp	Inulin	
	mg		min	ml/min	ml/min	ml/min	
RI	700	1	15	9.0	80	72	1.11
		2	16	4.9	70	79	0.89
		3	16	4.6	71	83	0.86
		4	14	6.3	83	98	0.85
		5	17	4.3	75	77	0.97
CN	800	1	15	13.1	110	103	1.07
		2	16	2.9	103	107	0.96
		3	15	3.9	98	110	0.89
		4	15	3.8	99	119	0.83
		5	15	4.7	100	125	0.80
MI	900	1	1	7.9	101	72	1.40
		2	17	4.4	96	71	1.35
		3	16	8.9	79	69	1.14
		4	16	3.2	72	78	0.92
		5	16	3.0	74	60	1.23
KG	1500	1	15	4.5	108	98	1.10
		2	1	3.7	117	112	1.04
		3	16	2.8	103	108	0.95
		4	18	1.5	90	97	0.93
		5	18	1.5	85	111	0.77
AL	1800	1	15	13.1	144	116	1.24
		2	15	9.8	117	86	1.36
		3	17	1.1	113	129	0.88
		4	16	2.3	112	135	0.83
		5	16	8.9	111	132	0.84
BR	2000	1	17	3.8	59	51	1.16
		2	16	3.5	63	55	1.15
		3	14	2.6	47	75	0.63
		4	16	1.2	50	59	0.85
		5	16	0.8	59	65	0.91
BN	2500	1	18	8.9	74	57	1.30
		2	16	3.8	75	60	1.25
		3	16	8.2	67	53	1.26
		4	17	1.0	64	36	1.78
		5	17	2.8	63	98	0.64

Case	Probenecid	Period		Urine flow	Clearance		Quotient Hyp / Inulin
		No	duration		Hyp	Inulin	
	mg		min	ml/min	ml/min	ml/min	
LC	500	1	17	2 3	96	85	1 13
		2	17	2 3	92	76	1 21
		3	19	2 9	86	72	1 19
		4	19	3 1	79	57	1 40
		5	19	5 2	87	79	1 10
VW	1500	1	16	9 9	78	59	1 32
		2	18	9 1	79	58	1 36
		3	23	3 7	66	50	1 32
		4	17	3 9	67	46	1 46
		5	17	4 2	70	53	1 32

Table XX Series A (Chapter VIII) Renogram parameters retention serum NPN and diagnosis in controls

Case	Sex Age (years)	Renogram						Chest curve Ret[3]	NPN	Diagnosis
		Right			Left					
		Up-take	Excr I[1]	Excr II[2]	Up take	Excr I	Excr II	%	mg %	
IC	M 22	1 053	1 252	1 162	1 053	1 357	1 167	59		Healthy
UM	M 22	1 069	1 178	1 115	1 083	1 286	1 167	55		"
GO	M 22	1 097	1 238	1 130	1 080	1 169	1 143	57		"
BL	M 22	1 139	1 480	1 220	1 089	1 459	1 276	57		"
SI	M 24	1 095	1 400	1 200	1 090	1 390	1 214	46		"
BB	M 24	1 063	1 153	1 154	1 031	1 220	1 242	59		"
OO	M 25	1 037	1 217	1 150	1 100	1 263	1 226	52		"
JM	M 25	1 159	1 152	1 179	1 119	1 218	1 222			"
VI	M 26	1 108	1 191	1 175	1 093	1 273	1 151	57		"
TI	M 28	1 059	1 342	1 188	1 045	1 414	1 261	52		"
CO	M 17	1 025	1 201	1 161	1 037	1 263	1 223	55	24	Cardiac neurosis
EP	M 18	1 073	1 353	1 259	1 058	1 383	1 288	54	23	Cephalalgia
JI	M 24	1 076	1 075	1 118	1 086	1 091	1 184	48	30	Cephalalgia
BJ	M 24	1 092	1 311	1 165	1 092	1 311	1 165	50	32	Rheum.arthrit
SG	M 27	1 065	1 384	1 263	1 049	1 370	1 305	58	33	Diss *sclerosis*
AO	F 30	1 167	1 403	1 241	1 140	1 473	1 257	46	28	Psychoneurosis
SN	M 32	1 101	1 352	1 255	1 087	1 389	1 180	53	26	Trigeminal neuralgia
PI	M 33	1 089	1 220	1 253	1 076	1 278	1 200	65	29	Paranoid reactions
SA	M 33	1 063	1 433	1 250	1 038	1 367	1 167	47	30	Gastric ulcer
SZ	M 34	1 108	1 449	1 216	1 074	1 338	1 213	60	33	Cephalalgia
AB	M 36	1 050	1 130	1 184	1 056	1 238	1 121	57	32	Duodenal ulcer
IO	F 39	1 051	1 362	1 184	1 069	1 329	1 197	56	28	Hyper-γ globulinem
LL	M 40	1 073	1 313	1 143	1 051	1 274	1 107	66	25	Gastric ulcer
MM	M 42	1 082	1 413	1 150	1 038	1 316	1 169	53	29	Acute gastritis
OB	M 43	1 034	1 191	1 099	1 089	1 270	1 125	60	26	Cephalalgia
EA	M 48	1 091	1 123	1 204	1 055	1 233	1 154	57	32	Cholelithiasis
HB	M 49	1 091	1 180	1 186	1 061	1 226	1 154	61	34	Lumbar disc degen.
ZB	F 50	1 0 5	1 265	1 360	1 036	1 246	1 326	56	31	Cholelithiasis
II	M 55	1 117	1 227	1 158	1 070	1 224	1 160	49	29	Duodenal ulcer
EK	M 57	1 042	1 330	1 266	1 087	1 444	1 324	66	24	Epilepsy
EN	M 7	1 057	1 202	1 167	1 065	1 192	1 099	67	34	Cephalalgia

[1] Excretion ratio 15/30
[2] Excretion ratio 5 15
[3] Retention per cent

Table XVI Reproducibility of renograms in 10 controls Two investigations (A and B) at 2 day interval

Case	Renogram								Chest curve Ret %	
	Right				Left					
	Uptake		Exer I		Uptake		Exer I			
	A	B	A	B	A	B	A	B	A	B
LK	1 042	1 031	1 266	1 316	1 087	1 099	1 324	1 373	66	53
LP	1 073	1 118	1 259	1 204	1 058	1 122	1 288	1 224	54	60
SN	1 101	1 085	1 255	1 196	1 087	1 073	1 180	1 231	53	55
II	1 117	1 100	1 158	1 193	1 070	1 063	1 160	1 176	49	53
SZ	1 108	1 153	1 216	1 153	1 074	1 114	1 213	1 208	60	54
BI	1 139	1 076	1 220	1 189	1 089	1 078	1 276	1 268	57	51
OB	1 034	0 941	1 099	1 122	1 089	1 073	1 125	1 151	60	56
I I	1 089	1 067	1 253	1 239	1 076	1 015	1 200	1 238	65	61
IO	1 051	1 000	1 184	1 173	1 069	1 057	1 197	1 150	56	63
SG	1 065	1 083	1 263	1 197	1 019	1 093	1 305	1 222	58	57
Mean	1 082	1 065	1 217	1 198	1 075	1 079	1 227	1 225	58	56

Table XVII Series B—G (Chapter VIII) Renogram parameters, retention and laboratory findings in cases of renal parenchymal diseases hypertension post nephrectomy and aplasia

Series B Chronic glom nephritis

Case	Sex Age (years)	Renogram				Chest curve Ret	Laboratory findings			
		Right		Left			NPN	Serum creatinine	Creatinine clearance[2]	Max conc
		Uptake	Exer I[1]	Uptake	Exer I	%	mg %	mg %	ml min	g ml
RS	F 47	0 931	1 101	0 971	1 121	70	44	1 00	123	1 016
TJ	M 33	0 923	1 029	0 956	1 021	68	33	1 15	89	1 013
SL	F 49	0 909	1 080	0 923	1 105	71	34	1 42	51	1 015
MI	F 44	0 951	1 157	0 964	1 118	55	46	1 11	42	1 021
AS	F 30	0 956	1 079	0 922	1 071	78	155	4 05	11	
VB	F 43		1 067		1 043	81	58	6 22	12	
BI	M 37	0 970	1 016	1 000	1 016	88	212	10 50	9	
GI	M 30	0 990	1 011	1 000	1 014	79	166	13 70		

Series C Chronic pyelonephritis

Case	Sex Age (years)	Renogram Right Uptake	Renogram Right Excr P	Renogram Left Uptake	Renogram Left Excr L	Chest curve Ret[a] %	NPN mg %	Serum creatinine mg %	Creatinine clearance[b] ml min	Max conc g ml
KS	F 19	1 043	1 063	1 105	1 000	65	25	0 66	133	
BM	F 46	0 968	1 167	1 089	1 321	57	32	0 82	112	1 026
AI	F 56	1 044	0 994	1 009	1 136	63	25	0 82	109	1 025
BZ	F 41	0 923	1 105	1 037	1 118	58	26	0 94	90	1 028
AT	F 52	0 968	1 083	1 000	1 109	71	38	1 28	65	1 026
RQ	F 52	1 000	1 026	0 976	1 094	69	23	1 33	59	1 012
MH	F 37	1 000	1 028	1 053	1 088	69	42	1 81	56	1 012
AB	F 54	0 915	1 060	0 953	1 061	72	35	1 74	43	
HH	F 52	0 944	1 036	0 918	1 025	83	78	2 07	35	
EP	F 61	0 939	1 033	0 935	1 031	75	48	2 40	35	
AO	M 55	0 967	1 010	1 020	1 033	72	50	2 89	30	
OA	M 64	1 000	1 091	1 000	1 057	64	60	2 84	19	
TB	M 65	0 951	1 031	0 976	1 056	75	69	4 00	23	1 009
MW	F 50	0 954	1 026	0 964	1 044	84	90	4 12		
AC	F 51	0 945	1 032	0 937	1 026	84	78	4 14	15	
SB	F 48	0 916	1 016	0 964	1 020		118	6 09	10	
SJ	F 36	0 937	1 010	0 961	1 070	69	142	11 00	9	1 007
LI	F 29	0 900	1 031		1 025	91	162			

Series D Polycystic disease

LL	M 21	1 160	1 197	1 000	1 075	60	32	0 96	143	1 010
ZN	F 41	1 088	1 016	0 950	1 045	82	11	1 39	72	
KJ	M 37		1 029	1 000	1 000	70		3 31	38	1 009
AC	M 38	1 000	1 051	1 000	1 058	78	199	12 00	5	

Series E Other parenchymal diseases

JY	M 54	1 026	1 153	1 053	1 098	62	30	0 80	153	1 027
HS	M 34	1 083	1 107	1 071	[illegible]	56	23	0 69	148	1 022
MI	M 22	1 038	1 149	1 049	1 163	62	28	[illegible]	137	1 028
BA	M 54	1 083	[illegible]	1 005	1 162	67	44	0 82		
HI	F 42	0 945	1 089	1 141	1 261	66	21	0 53	133	
BA	M [illegible]	1 086	[illegible]	1 018	1 328	64	32	1 08	117	1 035
AO	M 53	[illegible]	[illegible]	1 008	1 014	62	32	0 39	57	1 022
EM	M [illegible]	[illegible]	[illegible]	1 11	1 122	57	44	1 10	93	1 018
LA	F 19	1 108	1 107	1 074	1 234	72	25	0 76	83	1 023
BJ	M 29	[illegible]	1 058	[illegible]	[illegible]	71	46	1 13	80	1 016

Table XVI Reproducibility of renograms in 10 controls Two investigations (A and B) at 2 day interval

Case	Renogram								Chest curve Ret %	
	Right				Left					
	Uptake		Excr I		Uptake		Excr I			
	A	B	A	B	*A*	*B*	A	*B*	A	*B*
EK	1 042	1 031	1 266	1 316	1 087	1 099	1 324	1 373	66	53
LP	1 073	1 118	1 259	1 204	1 058	1 122	1 288	1 224	54	60
SN	1 101	1 085	1 255	1 196	1 087	1 073	1 180	1 234	53	55
II	1 117	1 100	1 158	1 193	1 070	1 063	1 160	1 176	19	53
SZ	*1 108*	1 153	1 216	1 153	1 074	1 114	1 213	1 208	60	54
BI	1 139	1 076	1 220	1 189	1 089	1 078	1 276	1 268	57	51
OB	1 034	0 941	1 099	1 122	1 089	1 073	1 125	1 154	60	56
FF	1 089	1 067	1 253	1 239	1 076	1 015	1 200	1 238	65	61
IO	1 051	1 000	1 184	1 173	1 069	1 057	1 197	1 150	56	63
SC	1 065	1 083	1 263	1 197	1 049	1 093	1 305	1 222	58	57
Mean	1 082	1 065	1 217	1 198	1 075	1 079	1 227	1 225	58	56

Table XVII Series B—G (Chapter VIII) Renogram parameters retention and laboratory findings in cases of renal parenchymal diseases hypertension post nephrectomy and aplasia

Series B Chronic glom nephritis

Case	Sex Age (years)	Renogram				Chest curve Ret²	Laboratory findings			
		Right		Left			NPN	Serum creatinine	Creatinine clearance³	Max conc
		Uptake	Excr I¹	Uptake	Excr I	%	mg %	mg %	ml min	g ml
RS	F 47	0 934	1 101	0 971	1 121	70	11	1 00	123	1 016
TJ	M 33	0 923	1 029	0 956	1 021	68	33	1 15	89	1 019
SE	F 49	0 909	1 080	0 923	1 105	71	34	1 12	51	1 01
MI	F 44	0 951	1 157	0 964	1 118	85	46	1 11	42	1 021
AS	F 30	0 956	1 079	0 922	1 071	78	155	1 05	13	
VB	F 43		1 067		1 043	81	88	6 22	12	
BI	M 37	0 970	1 016	1 000	1 016	88	212	10 50	9	
CL	M 30	0 990	1 011	1 000	1 014	79	166	13 70		

Table XVIII Series II (Chapter VIII) Renogram parameters and diagnosis in cases with acute ureteral obstruction (suspect or proven)

Case	Sex Age (years)	Renogram				Diagnosis
		Right		Left		
		Up take	Excr II[1]	Up take	Excr II	
MK	F 35	1 000	1 068	1 000	1 068	Acute cystitis
KI	M 46	1 047	1 075	1 065	1 116	Duodenal ulcer
BS	F 19	1 167	1 203	1 100	1 212	Acute cystitis
IP	F 62	1 022	1 178	0 989	1 193	Acute cholecystitis
AN	M 44	1 064	1 143	1 050	1 083	Ischemic lumbago
AC	F 28	1 000	1 250	1 000	1 320	Acute cholecystitis
KH	M 41	1 011	1 143	1 036	1 203	Abdominal pain
AH	F 38	0 904	1 079	0 914	1 108	Acute urethritis
NP	F 72	1 056	1 327	1 063	1 357	Diab mellitus + Herpes Zoster
ML	F 40	1 063	1 015	1 000	1 000	Acute pancreatitis
BP	F 14	1 057	1 319	1 038	1 370	Acute cystopyelitis
OA	M 25	1 095	1 132	1 059	1 200	After passage of stone
II	F 39	1 072	1 063	1 093	1 146	Right ov cyst.
MJ	F 56	0 943	1 119	1 140	1 297	Right renal aplasia
AS	M 33	1 081	0 870	1 103	1 138	Ureterolithiasis + Obstruction
II	M 35	1 036	0 888	1 064	1 132	" "
BR	M 28	1 032	0 925	1 021	1 184	" "
BW	M 52	1 026	0 549	1 050	1 261	" "
CN	M 52	1 061	0 946	1 020	0 912	" "
IL	M [illegible]	1 063	0 991	1 092	1 250	" "
CC	M 41	1 145	0 718	1 111	1 087	" "
SO	M 42	1 035	0 858	1 018	1 174	" "
TA	M 40	1 057	0 786	1 079	1 388	" "
CR	M 49	1 045	0 815	1 083	1 294	" "
IR	F 50	1 031	0 886	0 554	1 163	Obstruction
AB	F 21	1 119	0 863	1 049		
OR	F 50	1 023	0 934	1 023	1 262	"
CB	M 39	1 158	0 580	1 136	1 217	"
NW	M 40	0 955	1 196	1 053	0 509	Ureterolithiasis + Silent kidney
BO	M 25	1 051	0 854	1 074	1 241	" "
NM	M 70	1 055	1 097	1 071	0 918	Silent kidney
AI	M 48	1 061	0 842	1 057	1 149	Obstruction
LO	M 49	1 047	1 222	1 017	1 294	Obstruction(?)

[1] Excretion ratio 5 15

Case	Sex Age (years)	Renogram Right Uptake	Renogram Right Excr I[1]	Renogram Left Uptake	Renogram Left Excr I	Chest curve Ret[2] %	NPN mg %	Laboratory findings Serum creatinine mg %	Creatinine clearance[3] ml/min	Max conc g/ml
RM	M 54	1 059	1 068	1 032	1 016	65	35	0 85		
EB	F 54	0 992	1 075	0 982	1 081	73	48	1 17	45	1 016
CN	F 22	1 033	1 171	1 071	1 214	72	49	1 27		
AA	M 60	0 979	1 027	0 979	1 000	68	37	1 44	62	
KK	F 29	0 938	1 122		1 129	82	37	1 73	37	1 014
IP	M 52	0 953	1 043	0 933	1 091	82	38	2 13	42	1 008
EF	F 42	0 900	1 051	0 943	1 053	87	124	5 02	12	
EV	M 34	0 943	1 034	0 936	1 016	81	122			

Series F Hypertension

Case	Sex Age (years)	Renogram Right Uptake	Renogram Right Excr I[1]	Renogram Left Uptake	Renogram Left Excr I	Chest curve Ret[2] %	NPN mg %	Serum creatinine mg %	Creatinine clearance[3] ml/min	Max conc g/ml
BF	F 34	0 958	1 111	1 099	1 361	64	26	0 76	129	1 026
EJ	F 41	1 019	1 013	1 100	1 118	67	30	0 85	117	
YS	M 49	1 000	1 075	0 969	1 041	58	40	1 12	87	1 025
NN	*F 51*	*1 133*	*1 182*	*1 150*	*1 250*	*67*	*33*	*0 95*	*86*	
ÖN	F 52	1 025	1 168	1 023	1 119	63	25	0 76	83	
ÅH	F 45	1 000	1 000	0 984	1 036	90	66	1 71	51	
ÅN	M 56	0 971	1 111	0 985	1 115	68	38	1 76	54	
IG	F 30	1 037	1 013	1 047	1 068	73	36	1 53	43	1 017
JG	M 46	1 037	1 019	1 036	1 019	70	54	1 72	41	
AL	M 67	*0 857*	*1 067*	*0 850*	*1 032*	*77*	*104*	*3 10*	27	

Series C Nephrectomy or aplasia

Case	Sex Age (years)	Renogram Right Uptake	Renogram Right Excr I[1]	Renogram Left Uptake	Renogram Left Excr I	Chest curve Ret[2] %	NPN mg %	Serum creatinine mg %	Creatinine clearance[3] ml/min	Max conc g/ml
LA	*F 17*	*0 813*	*1 065*	*1 255*	*1 290*	*58*	*35*			
TO	F 41	0 897	1 066	1 070	1 172	65	26	1 18	90	1 016
ES	M 59	1 210	1 259	0 934	1 042	68	33	1 13	47	
WA	F 64	0 868	1 068	1 070	1 113	66	40	0 82	151	
HZ	F 64	1 070	1 112	0 928	1 119	74	38	1 25	49	1 011
OS	M 40	1 179	1 113	0 917	1 079	54	32			1 024

[1] Excretion ratio 15/30
[2] Retention per cent
[3] Corrected to hold for 1 73 m² body surface

Series C Chronic pyelonephritis

Case		
KS	*Ur*	R plump calyces and pelvis Parenchymal condens over spine
		L as right
		Horseshoe kidneys.
BM	*Ur*	R deform with depression of upper calyx group Small (10 5 × 5 5)
		L normal excr and morph (13 5 × 7 4)
	Ang	R normal ren art Small kidney
AL	*Ur*	R deform ren. pelv Normal excr
		L normal excr and morph
	Uget	R double renal pelvis and ureter Deformed pelvis
BZ	*Ur*	R fragm filling (11 × 6)
		L normal
	Uget	R all calyces deformed At cal x groups filling of peasized spaces
AT	*Ur*	R and L normal
IB	*Ur*	R and L normal.
	Aut	Chron pyelonephr with bilat contraction R 30 g. L 60 g R renal art narrow with scler plaque
HH	*Ur*	R and L poor excr Morph not evaluable
	Aut	Chron pyelonephr Small kidneys R 30 g L 110 g
[illegible]	*Ur*	R and L poor excr Calyces and pelvis dilated (Hydronephrosis) Bilat uret. catheterization produced no relief No signs of ureter stone
AO	*Ur*	R and L poor excr Dilat ren pelv Multiple concr Bilat. catheterization of ureter unsuccessful
OA	*Ur*	R and L poor excr Morph not evaluable
	Uget	L widened pelv Several cavities filled with contrast medium
	Lap	L nephrectomy
	Aut	Chron pyelonephr with contraction and pap necrosis
TH	*Aut*	*Chron pyelonephr L 100 g. R 180 g*
MW	*Lap*	Previous R nephrolithotomy Cross changes resembling chron pyelonephrit
AL	*Aut*	Chron pyelonephr with ren contraction R 37 g L 42 g.
SB	*Aut*	Bilat chron pyelonephr with extensive calc papillary necroses. R 40 g L 4[illegible] g
ST	*Aut*	Bilat chron pyelonephr with marked contraction R ectopic and hypoplastic. [illegible] g L 6[illegible] g
[illegible]	*Aut*	Chron pyelonephr with marked papillary changes with contraction Bilat. moderate hydroureter and hydronephrosis

Series D Polycystic disease

Case		
KI	*Ur*	R normal
		L large irregular lat outline and calyx group displaced
	Aort	R aberrant ren artery no stenosis
		L [illegible] nephrographic effect
	Lap	L kidney full of cysts. Congenital multiple cystic L kidney
ZS	*Ur* [illegible]	Enlarged kidneys with irregular outlines
	Aut	Enlarged kidneys with multiple cysts R 460 g L 510 g.
[illegible]	*Ur* [illegible]	Enlarged kidneys with irregular outlines
	Aut	Enlarged kidneys with cysts of various sizes.

Table XIX Series I (Chapter VIII) Renogram parameters retention and laboratory findings in cases of hydronephrosis

Case	Sex Age (years)	Renogram				Chest curve Ret [2]	Laboratory findings			
		Right		Left			N P N	Serum creatinine	Creatinine clearance[3]	Max conc
		Uptake	Excr I[1]	Uptake	Excr I	%	mg %	mg %	ml min	g ml
ÖP	M 33	1 167	1 074	1 158	1 281	57	27	0 68	144	1 023
IW	M 52	1 084	1 056	1 044	1 099	56	33	0 92	108	1 019
VP	M 45	1 087	1 106	1 108	0 886	50	35			
JW	M 57	1 011	1 035	1 115	1 136	70	32	0 88	100	1 021
DD	F 50	1 167	0 954	1 106	1 105	58	38			1 022
EL	F 33	1 014	0 968	1 034	1 088	67	26	0 96		
AM	M 55	1 062	1 314	1 038	0 957	66	31			1 014
KO	M 49	1 102	1 135	1 087	0 862	54	38	0 88	114	1 010
SS	F 50	1 077	0 978	1 077	1 111	63	36	0 63	94	1 026
SA	F 57	1 123	1 000	1 000	0 974	63		0 93	94	
LH	M 69	0 968	1 092	1 104	1 090	67	18	1 27	57	
FJ	M 69	0 903	1 034	1 098	0 961	58	44			1 011
IN	F 57	0 947	1 020	1 054	0 667		31	0 90	113	

[1] Excretion ratio 15/30
[2] Retention per cent
[3] Corrected to hold for 1 73 m² body surface

Table XX Series B—I (Chapter VIII) Results of roentgen examination laparotomy and autopsy Abbreviations R Right kidney L Left kidney Plain Plain roentgenography Ur Excretion urography Pyel Retrograde pyelography Ang Selective renal angiography Aort Aortography Lap Laparotomy Aut Autopsy

Series B Chronic glom nephritis

Case		
RS	*Aut*	Subchron glom nephrit with contraction R 110 g L 120 g (Bilat 11 [illegible])
TJ	*Ur*	R poor excr Morph not evaluable (12×5)
		L poor excr Morph not evaluable (11×)
	Aut	Chron glom nephrit with contraction R 80 g L 70 g
SL	*Ur*	R fragmentary filling Morph not evaluable (10×[illegible])
		L fragmentary filling Morph not evaluable (11×[illegible])
	Aut	Chron glom nephrit with bilat contraction R 65 g L 7[illegible] g
MI	*Aut*	Chron glom nephrit with bilat contraction R 60 g L 60 g
VB	*Aut*	Chron glom nephrit with bilat contraction R 75 g L 85 g
BI	*Aut*	Chron glom nephrit with contraction R 80 g L 100 g
GL	*Aut*	Chron glom nephrit with bilat contraction Small plaque R in ren art Stenosis (4 cm) at origin of L ren art — plaque

NN	Ur	R and L normal
ON	Ur	R normal (12 5×6)
		L normal (12×4)
	Aut	Nephrosclerosis Ren art normal Both kidneys small R 105 g L 80 g
MH	Ur	R poor excr Morph unevaluable Small (11×5)
		L poor excr Morph unevaluable (12 5×5)
AN	Ur	R normal excr and morph Small (11 2×5)
		L normal excr and morph Small (10 7×4 5)
IG	Ur	R and L normal
JG	Ur	R and L normal
	Aut	Nephrosclerosis R 110 g L 150 g Ren art normal bilat

Series C Nephrectomy aplasia

Case		
LA	Ur	R no excr No condens
		L normal excr Enlarged (17×17)
	Uret	R no excr No condens
		L normal
TO	Ur	*R no excr No condens*
		L normal excr and shape enlarged
	Uret	Catheter could be passed only 3—4 cm up right ureter
	Aut	(4 years after test) R No ren tissue demonstrable
		L chron and acute pyelonephritis
LN	Lap	Previous L nephrectomy Chron pyelonephritis
WA	Lap	Previous R nephrectomy Chron pyelonephritis with nephrolithiasis
HZ	Lap	Previous L nephrectomy Local interstit nephritis
OS	Lap	Previous L nephrectomy Chron pyelonephritis with nephrolithiasis

Series D Acute ureteral obstruction

Case		
AS	Ur	R no excr but condens in parenchyma Pinhead concr in ureteric orifice Obstruction with concr
		L no concr Normal excr
II	Ur	R no excr Concr 10×8 mm at level of L III
		L no concr Normal excr
	Lap	Urethrolithotomy
BH	Ur	R obstruction and dilat pelv and uret, filled down to concr in lower ureter Obstruction with concr
		L no concr Normal excr
BW	Ur	R after 12 min filling of moderately dilat pelv + ureter down to small intramural concr in ureter Obstruction with demonstrable concr
		L no concr Normal excr
CN	Ur	R no concr Normal excr
		L no excr after 35 min only condens in parenchyma Small concr at L IV Obstruction with ureter concr
EE	Ur	R retarded excr Filling of mod dilat ren pelv Ureter dilat down to concr at L II Obstruction with concr
		L no concr Normal excr

AG	*Plain*	Enlarged kidneys with irregular outlines Abundant calcifications
	Aut	Very larged cystic kidneys Pelvic deform and distended calyces R 3030 g L 2760 g

Series E Other parenchymal diseases

Case		
TY	*Ur*	R normal
		L double pelv and ureter (16×7 5)
HS	*Ur*	R normal excr
		L normal excr Numerous small papill calcif in both kidneys
MI	*Ur*	R and L normal
RA	*Ur*	R normal excr and morph (13×7 5)
		L normal excr and morph (16×6)
	Aort	Bilat normal Normal renal arteries L kidney larger than R
	Aut	R 200 g (13 5×6 5) Lower pole contains small concr and shows slight pyelonephritis Normal cortical and medullary zone L 1?0 g (11 5×6)
HE	*Ur*	R thin filling Morph not evaluable Small kidney
		L normal
	Aort	R small ren art Small kidney (6×2)
		L normal (15 6×7 5)
	Lap	Nephrectomy Hypoplastic kidney Chron pyelonephr
BA	*Ur*	R and L normal
AO	*Ur*	R and L normal
KM	*Ur*	R normal excr Small (12 5×5) Double pelv
		L normal excr and morph (14 5×7)
	Ang	R normal ren art
RM	*Ur*	R normal
		L retarded filling of large plump calyces
	Pyel	L plump, large calyces
	Lap	Nephrectomy Widespread tb and large cavern in lower pole Caseous necrosis
AA	*Ur*	Bilat horseshoe kidneys displaced to left Excr slightly retarded and thin No concr
KK	*Ur*	R and L Bilat low condensation Bilat malrotation Double ren pelvis and ureter
	Aort	Malrotation+anomalous ren art bilat
IP	*Ur*	R poor excr Morph not evaluable (11×5 5)
		L poor excr Morph not evaluable
LI	*Aut*	Chron and acute pyelonephr lesions and lesions of Kimmelstiel Wilson type R 290 g L 250 g

Series F Hypertension

Case		
BL	*Ur*	R small but of normal shape (11×5)
		L compens hypertrophy normal shape (15×7)
	Ang	R narrow ren art without certain stenosis
LJ	*Ur*	R fragmentary filling of pelvis and calyces Small (11×5)
		L compens hypertrophy normal morph (14×7 5)
YS	*Ur*	R and L normal

		L nori al
l	Ur	R norn al
		L signs of hydronephrosis
	I jel	R marked d lat of pelvis an l calyces l elv c junct on from anterior aspect of pelvis
	Lap	Verified hydronephrosis with ureteric stricture
W	*Ur*	R retar led exer l nture kidney occup ed by d lat pelvis an l d lat calyces and collecting ducts. (15 × 9)
		L normal
	Lap and tut	Large hydronephrotic sac Ren cancer (squamous ep thel al) Chron pyelo perinephrit
)D	*Ur*	R retarded exer and defect f ll ng of pelvis and ureter Concr in lower ureter (in cystocele) and o e concr in ureter widening
		I normal
	Lap	R Lithotomy
l	*Ur*	R retar led exer an l defect filling of pelvis w th d late l calyces No f lling of ureter Blad ler deformed by extravesical les on (abscess)
		I normal
	tut	(6 months later) Widesprea l n etastases and growth over both ureters Bilat obstru tion and signs of hydronephros s
VM	*Ur*	R normal
		L retar led exer l ill ng of widened pelvis Caudal part contained concr Ureter *springs fro n upper part of pelvis Obstruction at the ureteropelvic junction*
kO	*Ur*	R normal
		L retarded exer Large kidney with dilat pelvis and calyces Three concr in lower pole near caudal calyx group
	Lap	Nephrectomy (L) Hydronephrosis and chron pyelonephritis and nephrol th a s s
SS	*Ur*	R retar led exer Calyces and pelv s and collect. tubules slightly w der than norn al Ureter narrow at orig n (14 5 × 6 5)
		L norn al exer but son ewhat wide and plump calyces and pelvis (16 × 6 5)
SA	*Ur*	Bilat r tarde l exer D lat of pelv and calyses Drainage obstructed
l ll	*Ur*	R silent (6 × 3)
		L sligh tly enlarge l (14 8 × 6 4) Retarded exer Sac l ke pelv s and plu np cal ces D lat of ureter
	A g	An artery 2 n n w de running to aplastic kidney No fll n
	I g	Ir stat l ype tr pl s an l ureteral stricture
F l	*Ur*	R sile t
		L ret r le l exer and l f ct filling f dilat pelv an l calyces
	A l	Ir stat cancer R ki l ey s all and atrophic (50 g) Stenosis of R ureter B lat p lvis an l ureter
		L ki l ey weight 150 g D lat p lv and ureter Narrowing of ureter at orifice
IN	*l*	R nephrectomy
		L l tron p l rot c with arked d lat. f pelv and narrowing of uret and pelv
	I y l	I v rifie l hydron phros s

GG	*Ur*	R concr at level of process of L III Right ren obstruction and dilat of pelv and upper ureter No exer Caudal small concr Obstruction with demonstrable concr L no concr Normal exer
SO	*Ur*	R exer retarded and less than on L side Concr in lower R ureter Obstruction with concr L no concr Normal exer
FA	*Ur*	R retarded exer Dilat pelv and ureter Concr in lower ureter Obstruction with ureter concr L no concr Normal exer
GR	*Ur*	R retarded exer Dilat pelv, calyces and ureter Concr in lower ureter Obstruction with ureter concr L *no concr Normal exer*
LR	*Ur*	R slight enlargement No concr No exer but condens in parenchyma Signs of obstruction without demonstr concr L no concr Normal exer
AB	*Ur*	R no concr Entire ureter filled and wider than left Contrast persisted in lower ureter after exam Signs of obstruction without demonstr concr L no concr Normal exer
OR	*Ur*	R no concr Exer normal but slower than on other side Dilat pelv and ureter down to bladder Signs of obstruction without demonstr concr L no concr Normal exer
GB	*Ur*	R no concr Retarded exer Dilat calyces pelv and ureter Signs of obstruction without demonstrable concr L no concr Normal exer
NW	*Ur*	R no concr Normal exer L no exer Slight condens in parenchyma Concr at level of L I Obstruction with concr
BO	*Ur*	R slight exer Pelv and visible part of ureter dilat Small concr in ureter orifice Obstruction with concr L no concr Normal exer
NM	*Ur*	R no concr Normal exer Upper calyx group slightly displaced (tumour) L kidney not visualized
	Aut	(18 months after exam) Left sided nephrolithiasis Small adenoma in cortex of right kidney
AL	*Ur*	R no concr Exer retarded and only scanty contrast medium in calyses and pelvis Signs of obstruction without demonstr concr L no concr Normal exer
UÖ	*Ur*	R exer normal Ureter slightly wider than left No signs of obstruction L no concr Normal exer
	Ur	(3 weeks after exam) Right sided obstruction Concr in lower R ureter with dilat pelv and calyces ureter

In all other cases urography showed no concrement and normal excretion

Series 1 Hydronephrosis

Case		
ÖP	*Ur*	R retarded exer and widened pelvis and all calyx groups L normal
	Lap	Retroperitoneal abscess on R side
IW	*Ur*	R retarded exer Widened pelvis and ureter down to stenosis of last 1 cm of ureter

	L normal
Ur	R *normal*
	L signs of hydronephrosis
Pyel	R marked dilat of pelvis and calyces Pelvic *junction from anterior aspect of* pelvis
Lap	Verified hydronephrosis with ureteric stricture
Ur	R retarded exer Entire kidney occupied by dilat pelvis and dilat calyces and collecting ducts (15 5×9)
	L normal
Lap and Aut	Large hydronephrotic sac Ren cancer (squamous epithelial) Chron pyelo *perinephrit*
Ur	R retarded exer and defect filling of pelvis and ureter Concr In lower ureter (in cystocele) and one concr *in ureter widening*
	L normal
Lap	R Lithectomy
Ur	R retarded exer and defect filling of pelvis with dilated calyces No filling of ureter Bladder deformed by extravesical lesion (abscess)
	L normal
Aut	(6 months later) Widespread metastases and growth over both ureters Bilat *obstruction* and signs *of hydronephrosis*
Ur	R normal
	L *retarded exer Filling of widened pelvis Caudal part contained concr* Ureter springs from upper part of pelvis Obstruction at the ureteropelvic junction
Ur	R normal
	L retarded exer Large kidney with dilat pelvis and calyces Three concr in lower pole near caudal calyx group
Lap	Nephrectomy (L) Hydronephrosis and chron pyelonephritis and nephrolithiasis
Ur	R retarded exer Calyces and pelvis and collect tubules slightly wider than normal Ureter narrow at origin (14 5×6 5)
	L *normal exer but somewhat wide and plump calyces and pelvis (16×6 5)*
Ur	Bilat retarded exer Dilat of pelv and calyses Drainage obstructed
Ur	R *silent (6×3)*
	L slightly enlarged (14 8×6 4) Retarded exer Sac like pelvis and plump calyces Dilat of ureter
Ang	An artery 2 mm wide running to aplastic kidney No filling
Lap	Prostat hypertrophy and ureteral stricture
Ur	R silent
	L retarded exer and defect filling of dilat pelv and calyces
Aut	Prostat cancer R kidney small and atrophic (50 g) Stenosis of R *ureter* Bilat pelvis and ureter
	L kidney weight 150 g Dilat pelv and ureter *Narrowing of ureter at orifice*
Ur	R nephrectomy
	L hydronephrotic with marked dilat *of pelv and narrowing of* uret and pelv
Pyel	L verified hydronephrosis

CG	*Ur*	R concr at level of *process of L III* Right ren obstruction and dilat of pelv and upper ureter No excr Caudal small concr Obstruction with demonstrable concr
		L no concr Normal excr
SO	*Ur*	R excr retarded and less than on L side Concr in lower R ureter Obstruction with *concr*
		L no concr Normal excr
TA	*Ur*	R *retarded* excr *Dilat pelv and ureter Concr in lower* ureter Obstruction with ureter concr
		L no concr Normal excr
GR	*Ur*	R retarded excr *Dilat* pelv , calyces and ureter Concr in lower ureter Obstruction with *ureter* concr
		L no concr Normal excr
LR	*Ur*	R *slight* enlargement No concr No excr but condens in parenchyma Signs of obstruction without demonstr concr
		L no concr Normal excr
AB	*Ur*	R no concr Entire ureter filled and wider than left Contrast persisted in lower ureter after exam Signs of obstruction without demonstr concr
		L no concr Normal excr
OR	*Ur*	R no concr Excr normal but slower than on other side Dilat pelv and ureter down to bladder Signs of obstruction without demonstr concr
		L no concr Normal excr
GB	*Ur*	R no concr Retarded excr Dilat calyces pelv and ureter Signs of obstruction without demonstrable concr L no concr Normal excr
NR	*Ur*	*R* no concr Normal excr
		L no excr Slight condens in parenchyma Concr at level of L I Obstruction with concr
BO	*Ur*	R slight excr Pelv and visible part of ureter dilat Small concr in ureter *orifice Obstruction with concr*
		L no concr Normal excr
NM	*Ur*	R no concr Normal excr Upper calyx group slightly displaced (tumour)
		L kidney not visualized
	Aut	(18 months after exam) Left sided nephrolithiasis Small adenoma in cortex of right kidney
AL	*Ur*	R no concr Excr retarded and only scanty contrast medium in calyses and pelvis Signs of obstruction without demonstr concr
		L no concr Normal excr
UÖ	*Ur*	R excr normal Ureter slightly wider *than left No signs of obstruction*
		L no concr Normal excr
	Ur	(3 weeks after exam) Right sided obstruction Concr in lower R ureter with dilat pelv and calyces ureter

In all other cases urographs showed no concrement and normal excretion

Series I Hydronephrosis

Case

ÖP	*Ur*	R *retarded excr and widened pelvis and all calyx groups*
		L normal
	Lap	Retroperitoneal abscess on R side
IW	*Ur*	R retarded excr Widened pelvis and ureter down to stenosis of last 1 cm of ureter

REFERENCES

Abbassian A. Mich med Soc 61 957 1962
Abt A. F & Balkus V A J Urol (Balti more) 85 95 1961
Addis T & Shevky M C. Arch intern Med 30 559 1922
Anghileri L. J J nucl Med 4 155 1963
Bauer K M & Feine U Fortschr Ront genstr Beiheft zu 93 25 1960
Beall Jr A. C. Johnson P C Holman M R & Debakey M E Amer Surg. 29 317 1963
Becker G Schneider K W & Wolfer H J Strahlentherapie 122 416 1963
Berg N O Idbohrn H & Wendeberg B Acta radiol (Stockh) 50 285 1958
Bergstrom J Bucht H & Josephson B Scand J clin Lab Invest 11 71 1959
Berlyne N & Berlyne G M Acta med Scand 171 39 1962
Bianchi C. Minerva nucl 5 157 1961
Bianchi C & Toni P Minerva nucl 6 34 1962
— Minerva nefrol 10 116 1963
Bianchi C & Zampieri A Boll Soc ital Biol sper 37 260 1961
Billion H & Schlungbaum W Klin Wschr 33 1089 1955
Blaufox M D Campbell I C. Utz D C. & Owen Jr C A Proc Soc exp Biol. (N Y) 112 1069 1963 a
Blaufox M D Frohmuller H G W Camp bell J C Utz D C Orvis A L & Owen C A J surg Res 3 122 1963 b
Blaufox M D Orvis A L & Owen Jr C A Amer J Physiol 204 1059 1963 c.
Blaufox M D Sanderson D R Tauxe W N Wakim K G Orvis A L & Owen Jr C A Amer J Physiol 204 536 1963 d
Block J B & Burrows B A J Lab clin. Med 56 463 1960
Block J B Hine G J & Burrows B A. Circulation 22 913 1960 a
— J Lab clin. Med 56 110 1960 b
Block J B Rieselbach R E Bentzel C. J & Rall D P Proc. Soc exp Biol (N Y) 117 297 1964
Bodfish R L & Roberts N E. Amer J Roentgenol 84 861 1960
Boeminghaus H Arch klin Chir 171 109 1932
Bojesen E Acta physiol. scand 32 30 1954 a
— Acta physiol scand 32 129 1954 b
— Den tubulaere saltvandsresorption Christ treus bogtrykkeri Kobenhavn 1955
Boyd J D Morgan L A & Blum A Sth med J (Bgham Ala) 52 1 1959
Boyd J D & Murdock Jr H R Arch intern Med 109 654 1962
Brown F A Gelber R H Youkeles L H & Bennett L R J Amer med Ass 186 211 1963
Brun C J Lab clin Med 37 955 1951
Brun C Hilden T & Raaschou F Scand J clin Lab Invest. 1 348 1949 a
— J clin Invest. 28 144 1949 b
Burbank M K Hunt J C. Tauxe W N & Maher F T Circulation 27 328 1963
Burbank M K Tauxe, W N Maher F T & Hunt J C. Proc. Mayo Clin 36 372 1961
Burrows B A. Farmelant M H & Hine G J The radioactive diodrast renograms in renal atherosclerosis. In Hypertension. Recent advances Ed by Boest, A N and Moyer J H Lea & Febiger Philadelphia 1961 p 204
Cobb J Harper Hosp Bull. 19 91 1961
Coe F L & Burke G J nucl. Med 5 555 1964
Collins Jr J J, Pizak Jr L F Tanvako poulos S K & Wilson R E. Surg Forum 14 217 1963

Cases with renal disease in Chapter VIII (series B—C and I) The case numbers correspond to the following numbers in hospital records

Medical Department

RS	231/61
TJ	2062/59
SE	2341/63
MI	3056 60
AS	1471/58
VB	1907/61
BF	302 60
GE	2832 60
KS	3040/59
BM	3105/59
AL	133/61
BZ	3274/58
AT	918 61
MH	3428 60
IB	1539 63
HH	819/58
LP	992 63
AÖ	2734/62
OA	1312 62
EB	1452 61
AU	424/62
SB	696 60
SJ	164/59
LL	1113/58
KP	1448/58
ZN	134/60
KI	1975 61
AG	497/58
BE	647 60
LJ	3553/59
YS	331/64
NN	613/58
ÖN	1859/62
AH	1718/60
AN	2772/61
IG	2536/62
JG	2698/61
AL	685/58
TY	3238/59
HS	3308/63
MI	1063 60
RA	1249/61
HE	138 61
BA	1144/60
AO	197 61
KM	3659 60
EA	3354/58
HJ	399 61
EB	1326 61
GN	2390 64
AA	780 60
KK	3066 63
IP	2996 64
EF	2566/59
EN	415 61
TO	41 64
LS	2017 63
WA	1539 64
HZ	813/58
OS	3161 60
ÖP	727 58
IW	3355 60
JW	3315/59
AM	2137/60
SS	2256 62

Surgery Department

RM	3177/58
LA	4343/57
AP	718/58
DD	884/58
KO	4529/58
LH	4428/60
FJ	969/58
IN	3310/59

Department for Infectious diseases

MW	1919 60
RQ	2093/58

Department of gyn

EE	3338 60
SA	2895/60

REFERENCES

Abbassian A. Mich med Soc. *61* 957 1962

Alt, A. F & Balkus N A J Urol. (Baltimore) 85 95 1961

Addis T & Shevky M C Arch intern Med *30* 559 1922

Anghileri L J J nucl. Med *4* 155 1963

Bauer K M & Feine U Fortschr Rontgenstr Beiheft zu 93 25 1960

Beall Jr A C. Johnson P C Holman M B & Debakey M E Amer Surg 29 317 1963

Becker G., Schneider K W & Wolfer H J Strahlentherapie *122* 416 1963

Berg N O Idbohrn H & Wendeberg B Acta radiol (Stockh) *50* 285 1958

Bergstrom J Bucht H & Josephson B Scand J clin Lab Invest *11* 71 1959

Berlyne N & Berlyne G M Acta med Scand *171* 39 1962

Bianchi C. Minerva nucl *5* 157 1961

Bianchi C & Toni P Minerva nucl *6* 34 1962

— Minerva nefrol *10* 116 1963

Bianchi C & Zampieri A Boll Soc ital Biol sper *37* 260 1961

Billion H & Schlungbaum W Klin Wschr *33* 1089 1955

Blaufox M D Campbell J C Utz D C & Owen Jr C A Proc Soc exp Biol (N Y) *112* 1069 1963 a

Blaufox M D Frohmuller H G W Campbell J C. Utz D C Orvis A L & Owen C. A J surg Res *3* 122 1963 b

Blaufox M D Orvis A L & Owen Jr C A Amer J Physiol *204* 1059 1963 c

Blaufox M D Sanderson D R Tauxe W N Wakim K G Orvis A L & Owen Jr C A Amer J Physiol, *204* 536 1963 d

Block J B & Burrows B A J Lab clin Med. *56* 463 1960

Block J B Hine G J & Burrows B A Circulation *22* 913 1960 a

— J Lab clin Med *56* 718 1960 b

Block J B Rieselbach R E Bentzel C. J & Rall D P Proc. Soc exp Biol (N Y) *117* 297 1964

Bodfish R L & Roberts N E. Amer J Roentgenol 84 861 1960

Boeminghaus H Arch klin Chir *171* 109 1932

Bojesen E Acta physiol. scand *32* 39 1954 a

— Acta physiol scand *32* 129 1954 b

— Den tubulaere saltvandsresorption Christreus bogtrykkeri København 1955

Boyd, J D Morgan L. A & Blum A South med J (Bgham Ala) 52 1 1959

Boyd J D & Murdock Jr H R Arch intern Med *109* 654 1962

Brown I A Gelber R H Voukeles L H & Bennett L R J Amer med Ass *186* 211 1963

Brun C. J Lab clin. Med *37* 955 1951

Brun C. Hilden T & Raaschou F Scand J clin Lab Invest. *1* 348 1949 a

— J clin Invest. *28* 144 1949 b

Burbank M K Hunt J C. Tauxe W N & Maher F T Circulation 27 328 1963

Burbank M K Tauxe W N., Maher F T & Hunt J C Proc. Mayo Clin *36* 372 1961

Burrows B A Farmelant M H & Hine G J The radioactive diodrast renograms in renal atherosclerosis In Hypertension Recent advances. Ed by Boest, A N *and* Moyer J H Lea & Febiger Philadelphia 1961 p 204

Cobb J Harper Hosp Bull. *19* 91 1961

Coe F L & Burke G J nucl Med *5* 555 1964

Collins Jr J J Pizak Jr L. F Tamvakopoulos S K. & Wilson R E. Surg Forum *14* 217 1963

Davies D F & Shock N W J clin Invest *29* 496 1950

Dawborn J K & Doyle A E Med J Aust *50* 853 1963

Dejdar R Fortschr Röntgenstr *90* 196 1959

DeMaria W J A Krueger R Sanders A P & Baylin G J Amer J Roentgenol *90* 157 1963

DeMaria W Krueger R P Sanders A P James J M Politano V A & Baylin G J Proc Soc exp Biol (N Y) *104* 762 1960

Denneberg T Acta chir scand *118* 231 1959/60

— Nord Med *67* 759 1962

Denneberg T Ek J & Hedenskog I Acta med scand *170* 169 1961 a

Denneberg T Hansson L & Hedenskog I Acta med scand *166* 351 1960

— Acta chir scand *121* 465 1961 b

Denneberg T & Hedenskog I Nord Med *61* 812 1959 a

— Acta med scand *165* 61 1959 b

Dische S Caplan L & Kramer S Amer J Roentgenol *90* 149 1963

Doig A Lawrence J R Philp T Tothill P & Donald K W Brit med J *i* 500 1963

Dollery C T Proc roy Soc Med *53* 969 1960

Dore E K Taplin G V & Johnson D E Radiology *79* 923 1962

— J Amer med Ass *185* 925 1963

Edling N P G Edvall C A Helander C G & Pernow B Acta radiol (Stockh) *45* 85 1956

Egert H Wien Z inn Med *45* 353 1964

Elsom K A Bott P A & Shiels E H Amer J Physiol *115* 548 1936

Exler W R Clark M D Garman J E Rain R L & Meininger D E Radiology *78* 879 1962

Farmelant M H Lipetz C A Bikerman V & Burrows B A Amer J Surg *107* 50 1964

Farschidpur D & Schoknecht G Fortschr Röntgenstr *99* 697 1963

Feine U & Bauer K M Fortschr Röntgenstr *94* 623 1961

Frey K W Hör G & Heinze H G Nucl Med (Stuttg) *4* 249 1965

Friis T & Krogsgaard A R Acta med scand *176* 31 1964

Frohlich E D Fedor E E Leahy W V & Freis E D Med Ann D C *28* 324 1959

Garnett E S Brit J Urol 36 332 1964

Gasser G & Hawliczek F Wien med Wschr *112* 351 1962

— *Z* Urol *57* 29 1964

Gasser G Hawliczek F Rupprecht G & *Zita G* Wien klin Wschr *75* 749 1963

Gerbie A B & Flanagan C L Amer J Obstet Gynec *84* 1838 1962

Gerbie A B Flanagan C L & Woodbury J P Obstet and Gynec *18* 44 1961

— Amer J Obstet Gynec *83* 1138 1962

Gibson J G & Evans Jr W A J clin Invest *16* 317 1937

Gmelin R & Virtanen A J Acta chem scand *13* 1469 1959

Goldring W Chasis H Ranges H A & Smith H W J clin Invest *19* 739 1940

Gott F S Pritchard W H Young W R & Mac Intyre W J J nucl Med *3* 480 1962

Hansson R & Lindholm T Acta med scand *174* 611 1963

Harrow B R Amer J Roentgenol *75* 870 1956

Hauge A Oeff K & Gopel H Verh Dtsch Ges Urol (Sonderbd Z Urol) 1962 p 80

Haugen H N & Blegen E M Scand J clin Lab Invest *5* 67 1952

Hellmer H Acta radiol (Stockh) *16* 51 1935

Hennig K Woller P & Burkhardt G Radiolnol Radiother (Berl) *5* 303 1964

Herron G & Barbour B H Angiology *12* 352 1961

Heyrovsky A Clin chim Acta *1* 470 1956

Hilden T Diodrastclearance ved essentiell hypertension og glomerulonephritis Rosenhilde og Baggers Forlag København 1946

Hine G J Farmelant M H Cardarelli J A & Burrows B A J nucl Med *4* 371 1963

Hirakawa A & Corcoran A C J Lab clin Med *61* 795 1963

Hogeman O Acta med scand suppl 216 1948

Hoppe J O J Amer pharm Ass Sci ed *48* 368 1959

Hoppe J O Larsen A A & Coulston F J Pharmacol exp Ther *116* 394 1956
Horst W Rosler H Schneider C & Conrad B Dtsch med Wschr *86* 2485 1961
Hunt J C Harrison Jr E G Kincaid O W Bernatz P L & Davis G D Proc Mayo Clin *37* 181 1962 a
Hunt J C Tauxe W N Maher F T Greene L F Gifford Jr R W & Bernatz P L Amer J Cardiol *9* 134 1962 b
Hor G & Pabst H W Schweiz med Wschr *95* 445 1965
IAEA Acta radiol (Stockh) *58* 233 1962
Joekes A M & Rellan D R Lancet i 56 1965
Johnson Jr P C Driscoll T B & Nash F Cardiov Res Cent Bull 2 80 1964
Johnson Jr P C & Odom D D J nucl Med *5* 180 1964
Johnson D L Taplin G V Dore E K & Hayashi J J nucl Med 2 8 1961
Keith N M Wagener H P & Barker N W Amer J med Sci *197* 332 1939
Kimbel K H Diskussion zum Vortrag Schlungbaum Radioaktive Isotope in Klinik und Forschung Bd II Urban & Schwarzenberg Munchen Berlin 1956 p 150
Tierexperimentelle Grundlagen der Nierenfunktionsprufung mit radioaktiv markierten Kontrastmitteln Radioaktive Isotope in Klinik und Forschung Bd V Urban & Schwarzenberg Munchen Berlin 1963 p 245
Kimbel K H & Borner W Naunyn Schmiedebergs Arch exp Path Pharmak 226 205 1955
Kinter W B & Pappenheimer J R Amer J Physiol 185 391 1956
Klapproth H J Hirakawa A & Corcoran A C J Urol (Baltimore) *87* 17 1962
Knoefel P K & Huang K C J Pharmacol exp Ther *117* 307 1956
Knoefel P K Huang K C & Jarboe C H J Pharmacol exp Ther *134* 266 1961
Koecke K & Bauer H Fortschr Rontgenstr Beih 98 127 1963
Kretschmar I H & Greene W M J Urol (Baltimore) 90 110 1963
Krogsgaard A R & Friis T Acta med scand *176* 17 1964
Krueger P Sanders A P DeMaria W & Baylin G J Amer J Roentgenol *86* 819 1961
Kutka M Gilardi R & Semprebene L Int J appl Radiat *15* 213 1964
Langecker H Harwart A & Junkmann K Naunyn Schmiedebergs Arch exp Path Pharmak 222 584 1954
Larsen A A Hoppe J O McAuliff J P McChesney E W Grant J I & Coulston F Data in the files of Sterling Wintrop Research Institute 1954
Lasser E C Farr R S Fujimagari T & Tripp W N Amer J Roentgenol *87* 338 1962
Laurell C B Laurell S & Skoog N Clin Chem 2 99 1956
Lawrence J R Doig A Knight I C S & Tothill P Brit med J *i* 504 1963
Lindahl O & Josephson B Acta med scand *120* 195 1945
Lindell S I Diskussion zu den Vortragen Scheer und zum Winkel Radioaktive Isotope in Klinik und Forschung Bd V Urban & Schwarzenberg Munchen Berlin 1963 p 297
Lindgren P Acta radiol (Stockh) 56 423 1961
Lindgren P & Tornell G Acta radiol (Stockh) *49* 425 1958
Loken M K Staab E V Vernier R L & Kelly W D J nucl Med 5 807 1964
Lotspeich W D Metabolic aspects of renal function Charles C Thomas Springfield Ill 1959
Lowman R M Shapiro H & Newman H R J Canad med Ass 73 265 1955
Løken F Scand J clin Lab Invest *6* 395 1954
Magnusson G Acta med scand *166* 35 1960
— Acta med scand suppl 378 1962
Maxwell M H Amer J Cardiol 9 196 1962
Maxwell M H Gonick H C Wiita R & Kaufman J J New Engl J Med *270* 213 1964
Maxwell M H Rockney R E Winter C C & Kleeman C R Circulation 22 785 1960
McChesney E W & Hoppe J O Amer J

Roentgenol *78* 137, 1957

Meade R C & Shy, C M J Urol (Baltimore) *86* 163, 1961

The Merck index of chemicals and drugs 6th ed, 1952 Merck & Co, Inc, Rahway, N J USA

Meschan I, Deyton E W Schmid H E & Watts F C Radiology *81* 974, 1963 a

Meschan I Hosick T, Schmid H & Watts F C J nucl Med *4* 70, 1963 b

Meschan I Schmid H E, Watts F C & Witcofski R Radiology *81* 437, 1963 c

Minami, F, Ando K Ohori T & Machida T Urol int (Basel) *12* 148 1961

Moell, H Acta radiol (Stockh) suppl *206* 1961

Montandon A Wenger P & Roth H W Schweiz med Wschr *92* 35 1962

Moore T D & Mayer, R F Sth med J (Bgham Ala) *48* 135 1955

Moore F D Olesen K H McMurrey, J D Parker H V Ball M R & Boyden, C M *The body cell mass and its supporting environment* W B Saunders Company Philadelphia London 1963

Morris A M Elwood C Sigman E M & Catanzaro A J nucl Med *6* 183 1965

Moses J J Prentiss R J Moore I V Kiser, J R Belhard W F Tighe W J & Whitehead E L J Urol (Baltimore) *85* 679 1961

Newman E Kattus A A Genecin A Calkins L & Murphy J Johns Hopk Hosp Bull *84* 135 1949

Nordyke R A Tubis M & Bladh W H J Lab clin Med *56* 438 1960

O Conor, Jr V J Libretti J V & Grayhack J T J Urol (Baltimore) *86* 276 1961

Oeff K Gopel H & Hauge A Klin Wschr *39* 1298 1961

Oeff K, Lorenz W & Scholz A Dtsch Ges inn Med 69 Kongr 1963 p 749

Oeser H & Billion H Fortschr Röntgenstr *76* 431 1952

Olsson O & Jonsson G Roentgen examination of the kidney and the ureter Handbuch der Urologie Bd V/I J Springer Berlin 1962

Pabst H W Münch med Wschr *107* 1581 1965

Parker R H & Beierwaltes W H J nucl Med *1* 41 1960

Pedersen, F Andersen E Binder, C & Nielsen A A Ugeskr Laeg *126* 727 1964

Pedersen, F & Sörensen A H Ugeskr Laeg *126* 731, 1964

Pircher F J Carr Jr E A & Patno M E J nucl Med *4* 117 1963

Poker N Marshall V F, Becker D V & Evans, J A Amer J Roentgenol *84* 866 1960

von Porat, B Acta med scand suppl 256 1951

Quinn III J L Amer J Roentgenol *90* 135 1963

Quinn III J L Meschan I Blake, D D & Witcofski R L Radiology *78* 266 1962

Rappaport F & Eichhorn F J Lab clin Med *32* 1034, 1947

Riggs D S The mathematical approach to physiological problems Williams & Wilkins Company Baltimore 1963

Roddick Jr J W Gerbie A B & Flanagan C L Amer J Obstet Gynec. *88* 97 1964

Ross J C Edwards L C. Kulke W & Haggart B G Brit J Urol *35* 394 1963

Roth H W Montandon A Scherer J Stroun J & Wenger P Isotopennephrographie mit Hypaque und Hippuran bei Mensch und Tier Radioaktive Isotope in Klinik und Forschung Bd V Urban & Schwarzenberg Munchen Berlin 1963 p 272

Roth H W Movarrekhi H & Wenger P Schweiz med Wschr *90* 286 1960

Scheer K E & zum Winkel K Nucl Med (Stuttg) *1* 219 1960

Schlungbaum W Fortschr Med *23* 625 1961

— Fortschr Röntgenstr *96* 795 1962

Schlungbaum W & Billion H Strahlentherapie Sonderbd *36* 145 1956 a

— Klin Wschr *34* 633 1956 b

Scholz A Hauge A & Oeff K Dtsch med Wschr *89* 1011 1964

Schwarz I D & Madeloff M S J Urol (Baltimore) *87* 249 1962

Schutterle G & zum Winkel K Dtsch Ges. inn Med 69 Kongr 1963 p 761

Scott Jr R & Quesada L M Sth med J

(Bham Ala) 55 588 1962
Serrallo M Minerva nucl 6 219 1962.
Serrallo M Grayhack J T & Earle D P Arch intern. Med *103* 851 1959
Shy C. M & Meade R C. Marquette med Rev *26* 139 1961
Sieberth H G & Mertz D P Z ges exp Med *135* 330 1965
Sigman L M Kutzman N & Brown B J Urol (Baltimore) *90* 28 1963
Smith H W The kidney Structure and function in health and disease New York Oxford University Press 1951
Smith H W Finkelstein N Aliminosa L Crawford B & Graber M J clin. Invest *24* 388 1945
Smith H W Goldring W & Chasis H J clin Invest *17* 263 1938
Smith W W & Smith H W J biol Chem *124* 107 1938
Spencer C C Callendine Jr G W & Vincent D J J Lab clin Med *57* 360 1961
Spencer R P & Sigman L Experientia (Basel) *19* 201 1962
Spjut H J & Nicolai C H J Urol (Baltimore) *85* 115 1961
Squire L. I & Schlegel J U Radiology *73* 849 1959
Staehler W Bauer K M & Feste U Med Welt (Stuttg) 1 116 1963
Stewart B H & Haynie T P J Amer med Ass *180* 454 1962
Stewart B H de Weese M S Conway J & Correa Jr R J Arch Surg *85* 617 1962
Stokes J M Conklin J W & Huntley H C J Urol (Baltimore) *87* 630 1962
Stokes J M & Ter Pogossian M M J Amer med Ass *187* 20 1964
Straffon R A & Garcia A M J Urol (Baltimore) *83* 774 1960
Swick M Klin Wschr *8* 2087 1929
Surg Gynec Obstet *56* 62 1933
Saterborg N E Acta radiol (Stockh) *53* 433 1960
Taplin G V Nucleonics *22* 58 1964
Taplin G V Dore L K & Johnson D L. J nucl Med 4 401 1963
Taplin G V Meredith Jr O M Kade H & Winter C. C. J Lab clin Med *48* 886 1956.
Tauxe W N Proc Mayo Clin *36* 684 1961
Tauxe W N Burbank M K Maher F T & Hunt, J C. Proc. Mayo Clin. *39* 761 1964
Tauxe W N., Hunt J C. & Burbank M K Amer J clin Path *37* 567 1962
Trinkle J K & Kiser W S J Urol (Baltimore) *91* 199 1964
Wall C A & Whalen T J J Amer med Ass. *192* 1125 1965
Wallingford V H Decker H G & Kruty M J Amer chem Soc. 74 4365 1952
Wax S H Frank I N & McDonald D F J Urol (Baltimore) *88* 433 1962
Wax S H & McDonald D F J Amer med Ass *179* 140 1962
— J Urol (Baltimore) *92* 409 1964
Wedeen R P Goldstein M H & Lewitt M F Amer J Med. *34* 765 1963
Weissenstein H & Felkl, K Nucl Med (Stuttg) *4* 391 1965
West R T & Nordyke R A West. J Surg *71* 1 1963
Whitley J E. Witcofski R L Felts J H & Meschan I Surgery *50* 673 1961
Whitley J Witcofski R L. Quinn III J L & Meschan, I Radiology *78* 414 1962
Wieland T & Pfleiderer G Angew Chem *67* 257 1955
Willenbrink J & Kimbel K H Naunyn Schmiedebergs Arch exp Path Pharmak *236* 16 1959
Williams J L & Fisher M Brit J Surg *51* 875 1964
Wisenbaugh P E Clark I L Wills N L & Jeliffe R W Amer Heart J *69* 655 1965
Winter C. C. J Urol. (Baltimore) *76* 182 1956
— J Urol (Baltimore) *78* 107 1957
— Amer J Roentgenol. *82* 862 1959
— J Urol (Baltimore) *83* 313 1960
— J Urol (Baltimore) *85* 683 1961
— Radioisotope renography A kidney function test performed with radioisotope labelled agents. Williams and Wilkins Company Baltimore 1963
Winter C. C., Maxwell M H., Rockney R E & Kleeman C. R J Urol (Baltimore) *82*

674 1959
Winter C C Nordyke R A & Tubis M J Urol (Baltimore) *85* 92 1961
Winter C C & Taplin G V J Urol (Baltimore) *79* 573 1958
Witcofski R L Whitley J E, Meschan I & Painter W E Radiology *76* 621 1961
Woodruff, M W & Malvin R L J Urol (Baltimore) *84* 677, 1960
Wossidlo D Z Urol Sonderheft 1952 p 128
Wulff H B Acta radiol (Stockh) suppl 42 1936
zum Winkel K Klin Wschr *39* 757, 1961
— Nierendiagnostik mit Radioisotopen Georg Thieme Verlag Stuttgart 1964
zum Winkel K Becker J & Scheer K L Strahlentherapie *116* 489 1961 a
zum Winkel K Becker J & Schubert G Strahlentherapie *119* 4 1962
zum Winkel K & DeMaria D Nucl Med (Stuttg) *1* 397 1961
zum Winkel K & Scheer K E Chirurg *31* 487 1960
zum Winkel K Scheer K E & Becker J Med Welt (Stuttg) 1 576 1960 a
— Z Urol *53* 535 1960 b
zum Winkel K Scheer K E & Kazim I Brit J Urol *34* 241 1961 b
zum Winkel K Schutterle G & Scheer K L Dtsch med Wschr *86* 1751 1961 c.

ACTA MEDICA SCANDINAVICA

SUPPLEMENTUM 444

JAUNDICE DURING PREGNANCY

WITH SPECIAL EMPHASIS ON RECURRENT JAUNDICE DURING PREGNANCY AND ITS DIFFERENTIAL DIAGNOSIS

BY

URS PETER HAEMMERLI

ACCOMPANIES VOL. 179

STOCKHOLM 1966

FOREWORD

During the years 1959 to 1964 we had the occasion to observe personally 5 patients with recurrent intrahepatic cholestasis of pregnancy and to follow them through multiple gestations These cases, together with a sixth case discovered in the hospital files, have been described in detail elsewhere (Haemmerli and Wyss) The perusal of the literature during the preparation of that manuscript soon revealed, that not all cases of recurrent jaundice *during* pregnancy could be due to recurrent jaundice *of* pregnancy i e. the cholestatic form It also became apparent that an attempt at a differential diagnosis of recurrent jaundice during pregnancy has never been made and that many conflicting statements in the literature on recurrent jaundice during pregnancy originate in the lack of clear definitions

The present paper attempts to fill this need In order to achieve our purpose it has become necessary to present in a first part a brief review of the changes in so called liver function tests during uncomplicated pregnancy and in a second part a general review of jaundice during pregnancy The third and main part will be devoted to a description and definition of recurrent intrahepatic cholestasis *of* pregnancy on the basis of all verified cases in the world literature including our own patients and to a description of all other disorders which may present as recurrent jaundice *during* pregnancy We believe that the literature in the third part is as complete as it possibly can be The first two parts had to be somewhat restricted in content, and while they—as we hope—will present a fair summary of the present state of knowledge not all papers covering the respective topics could be included

Our interest in jaundice during pregnancy has been stimulated during our work as medical consultant and gastroenterologist to the Department of Obstetrics Zurich University Hospital and I wish to gratefully acknowledge the continuing help and encouragement received from its chief Professor E R Held his co-worker Dr H I Wyss and from my chief Professor P H Rossier, head of the Department of Medicine Zurich University Hospital

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I The liver in normal pregnancy

The liver performs its function well during gestation. Tests and laboratory determinations usually employed to evaluate liver function and liver disease deviate however not infrequently from the normal in healthy pregnant women These disturbances are rarely severe surpassing the accepted upper limit of normal for non pregnant females only slightly They are on the main more common in the later weeks of gestation and are usually rectified after delivery

The knowledge of the physiological derangements is important for any physician evaluating a women with jaundice during pregnancy In the non pregnant state there exists for every test an accepted division line between a normal and a pathological result It is probably wise to follow Friedberg s suggestion to form a third intermediary group of test results in pregnant women those lying above the upper limit of normal for non pregnant subjects but lying below the highest observed values in uncomplicated pregnancies As only a certain percentage of pregnant females surpass the non pregnant norm this intermediary group may then be truely normal or may have a pathological significance

Reviews of liver function in uncomplicated pregnancy have been attempted by Holmer 1927 Vignes 1935 Dietel 1936 Williams 1952 Dieckmann 1952 Lichtman 1953, Thorling 1955, Hoyneck Van Papendrecht 1957, Richman 1960 and Friedberg 1960 1962, 1963 A fair summing up has been made by Cross in 1929 The liver is the largest, the most abused the most neglected, one of the most important, and the least understood organ of the body'

Liver palpation

Palpation of the liver can be difficult in the later weeks of gestation, when the liver may be forced upwards, backwards and to the right by the enlarged uterus A normal liver is rarely palpable towards term When it is felt liver disease or congestive heart failure should be suspected

Spider angiomas and palmar erythema

Bean et al carefully examined all women in a prenatal clinic during one year They found spider angiomas in 66 6 % of 484 white and in 11 4 % of 759 negro pregnant females The control incidence was 12 % among 58 non pregnant white females with children and 14 9 % among 295 white soldiers In the same pregnancy groups 62.5 % of white and 35 % of negro females had palmar erythema There was an overlap in the occurrence of spider angiomas and palmar erythema in about two thirds of each group Spider angiomas occurred as early as the second month of gestation

Hemoglobin and serum iron

Young et al demonstrated a fall in hemoglobin levels during pregnancy in 219 serially examined patients Hoch et al found in addition a fall in serum iron levels, but could not demonstrate a correlation between hemoglobin and serum iron In a more detailed study of 176 patients Niesert observed an average fall in hemoglobin from 95 % to 85 %, a fluctuating but on the whole constant serum iron level, an increase in total iron binding capacity and a corresponding fall in iron saturation Ikonen found the serum iron levels widely dispersed (range 59—294 microgm per 100 ml) in his 84 normal pregnancies

Anemia during the last trimester of pregnancy is generally considered to be present only when the hemoglobin level falls to below 10 gm per 100 ml The fall in hemoglobin and hematocrit is explained by the rise in plasma volume which is only partially compensated by a minor rise in red cell volume (Tysoe and Lowenstein)

Total leucocyte and differential count

Kuvin and Brecher found a normal white cell count and morphology in only 46 % of 88 pregnancies 20 % have an increase of total white cells above 10 000 per cu mm and counts of 15 000 per cu mm are still considered normal in the last trimester Abnormal differential counts are also common There is an increase in both segmented and nonsegmented neutrophils Not infrequently myelocytes and metamyelocytes may be seen which are not necessarily part of the "shift to the left

Prothrombin time

The prothrombin time remains normal in all cases of uncomplicated pregnancy (Ikonen)

Urinary bile components

Although most textbooks state that urinary urobilinogen and urobilin may be increased in the later stages of pregnancy, few exact studies have been performed. Merletti in 1902 observed as a rule a two to three fold increase of urobilin in the last trimester In normal pregnancies at term Arfwedson found 'pathological bile components' in 5 % of 100 patients, Dieckmann et al a positive urine bilirubin test in 14.5 % of 85 patients and Labo et al a positive urine urobilin test in 8 3 % of 75 patients On the other hand Cross reported bilirubin urobilin and urobilinogen tests to be all normal in 61 uncomplicated pregnancies

Serum bilirubin

Total serum bilirubin levels were reported to be normal during uncomplicated pregnancy by Cross in 61 patients, by Cantarow et al in 34 patients by Dieckmann et al in 85 patients, by Wetstone et al in 56 patients and by Thorling in 202 patients The latter author gives a mean of 0 3 mg per 100 ml with a range of 0 1 to 1 1 mg per 100 ml

Other authors report a mild serum bilirubin increase in a small percentage of normal pregnancies, e g Eufinger and Bader Ikonen found bilirubin levels of 1 0 to 2 0 mg per 100 ml in 2 % of 100 patients, Arfwedson in 6 % of 100 patients and McNair and Jaynes in 4 3 % of 564 patients Among Friedberg s 120

with a sharp incidence rise between the 2nd and 5th month and but a slow rise thereafter During pregnancy the single spider angioma may increase in size and new ones may appear in the second and third trimester Most disappear after delivery

Bean et al 's study is the result of a meticulous search for these skin changes It is not surprising that they are usually not noted by the more hasty observer and that they are rarely mentioned in obstetric textbooks

Histological changes in liver biopsies

In 1907 Hofbauer described as typical histological changes of the liver during pregnancy centrolobular fatty degeneration, decreased glycogen content, centrolobular bile stasis and ectasia of the centrolobular veins and capillaries He coined the term "Schwangerschaftsleber" His opinion was based on 4 postmortem examinations of pregnant women dying at term three from pulmonary emboli and one from exsanguination during delivery This concept was challenged in 1910 by Schickele but still kept appearing in the literature up to 1945 It is now clear that Hofbauer's observations were due to unrelated and terminal pathology in his 4 cases

In 1945 Ingerslev and Teilum performed liver biopsies in 17 females during delivery They found some variation in the size of liver cells and nuclei occasionally small lymphocytic infiltrations in the portal spaces, and sometimes a mild vacuolar accumulation of fat in the centrolobular area which was more pronounced than in their 6 nonpregnant controls Otherwise the liver histology was considered normal In 1947 Nixon et al biopsied 9 pregnant females and found only minor nonspecific histological changes These consisted in an occasional variation in the shape of liver cells, in an increase in the number of large nuclei, in some irregularities of the nuclei, and in an increased glycogen content of the cytoplasm In the same year Dietel obtained 31 surgical liver biopsies during pregnancy and found only a slight increase in the number of binucleated liver cells when compared with 50 non pregnant controls

All these authors agree that histological liver changes during pregnancy are minor and nonspecific and that a "Schwangerschaftsleber" does not exist

Liver blood flow

Liver blood flow is within the normal range in pregnancy Using the bromsulfalein technic and hepatic vein catheterization Munnell and Taylor found a mean liver blood flow of 1,554 ml per minute per 1 73 sq m of body surface in 15 pregnant females (range 1 075—2,465) compared to a mean of 1 548 ml per minute (range 1,177—1,900) in 15 nonpregnant controls

This is noteworthy because in pregnancy plasma volume and blood volume rise by 50 to 60 % and cardiac output increases by 30 to 50 % reaching a maximum in the 7th pregnancy month and returning to normal towards term (Tysoe and Lowenstein) Liver blood flow comprises 35 % of cardiac output in nonpregnant females and only 28 % of cardiac output in pregnancy The excess blood volume is shunted through the placenta

TABLE I Mean serum alkaline phosphatase activity during uncomplicated pregnancy

Alk phosphatase method and units	Normal range	Month of gestation 5	6	7	8	9	10	Number of pat	References
King Armstrong	1 - 14	7 4	7 8	7 9	10 7	12 1	13 6	78	Mukherjee
Bodansky	1 – 4	—	2 9	3 2	3 6	4 7	5 9	300	Bodansky et al.
Buch & Buch	1 – 8	3 7	3 9	4 9	5 8	8 6	9 5	1/9	Thorling
Bessey Lowry	0 8 2 3	1 4	1 6	1 7	2 3	3 1	2 7	43	Beck and Clark
Roberts	0 – 6	3 0	3 3	4 7	8 3	10 4	12 5	201	Meranze et al
Vermehren	17 – 66	68	64	100	134	143	152	139	Vermehren
Shinowara Jones-Reinhart	2 – 9	3 2	3 4	3 9	4 7	11 4	11 8	239	Friedman et al

month differ markedly when compared to the respective upper limit of normal The upper limit of normal is just reached with the King—Armstrong method It is slightly surpassed in the Bodansky Buch and Buch Bessey—Lowry and the Shinowara—Jones—Reinhart technique and reaches twice the upper limit of normal with the Roberts and the Vermehren method In individual women there may be little or no rise in others the increase towards term is marked (Thorling Beck and Clark) In the last two months 28 % surpassed 14 King—Armstrong units (Mukherjee) 41 % 20 King—Armstrong units (Young et al) 11 % 5 5 Bessey—Lowry units (Ikonen) 60 % surpassed 4 and 20 % 6 Bodansky units (Bodansky et al)

Alkaline phosphatase rises again during labor especially in the second stage In 15 serially examined women Mukherjee found mean alkaline phosphatases of 13 5 King—Armstrong units during the tenth month 13 7 units during the first stage of labor 18 2 units during the second stage of labor and 11 4 units on the 4th day after delivery The underlying mechanism of this alkaline phosphatase rise is not clear Generally it is felt to represent a physiological response to the demands made by the foetus during the last 3 months of gestation (Mukherjee) Beck and Clark believe that the excess phosphatase originates in the placenta They found no increase of the alkaline phosphatase component which can be inhibited by sodium taurocholate and which is presumed to stem from bone and kidney Alkaline phosphatase activity falls to pre pregnancy levels within 4 to 6 weeks after delivery

Serum transaminases and other serum enzymes

Serum glutamic oxalacetic transaminase remains normal throughout pregnancy (Mason and Wroblewski extensive study Borglin 34 cases, West and Zimmerman 70 cases, Knutson et al 100 cases, Crisp et al. 30 cases, Stone et al 71 cases Kubli 32 cases Friedman et

patients 15 % had a serum bilirubin of 1 0 to 1 5 mg per 100 ml and 1 7 % of more than 1 5 mg per 100 ml Among Dieckmann et al 's 85 patients with normal total bilirubin levels 20 % had an increase of the 1 minute direct reacting fraction

Neither Thorling nor McNair and Jaynes found a correlation between bilirubin levels and stage of gestation There is no rise toward term, as does occur with alkaline phosphatase activity

However, intravenous bilirubin tolerance tests are often impaired with advancing pregnancy Among pregnant females with normal serum bilirubin levels Kaufmann found an increased bilirubin retention in 7 of 16 during the second half of pregnancy, Nurnberger in 13 of 25 toward the end of pregnancy, Soffer in 1 of 11 during the first trimester and in 9 of 10 during the second and third trimester, Sullivan et al in 1 of 11 during the first half and in 15 of 47 during the second half of pregnancy Soffer examined 10 patients twice and found in 7 a definite increase in bilirubin retention during the latter part of gestation No studies of this nature have been performed since 1934

Bromsulfalein retention

Normal bromsulfalein excretion during pregnancy was found in two early reports in 67 (Siegal) and 61 patients (Cross) except during labor A slightly increased bromsulfalein retention was not infrequently reported towards term in later series Thus, Christhilf and Bonsnes found a 5—10 % retention at 45 minutes in 6 of 36 and Cantarow et al in 7 of 34 pregnancies at term In the third trimester Labo et al observed a 7—12 % retention in 7 of 75 pregnancies while Friedberg (1962) found a retention of more than 5 % in 11 % and of more than 10 % in 3 7 % of his patients Mean bromsulfalein retention was reported by Wilken as 4 % during months 4—6, as 9 7 % during month 10 and as 16 3 % during labor He ascribed this increased retention during labor to a temporarily reduced liver blood flow

Combes et al, using the prolonged bromsulfalein infusion technique of Wheeler, found in 15 women in the second half of pregnancy a mean increase in relative hepatic storage capacity (S) of 122 % and a mean decrease of maximal excretory capacity (T_m) of 27 % while the results in the same women in the first half of pregnancy were no different from 10 normal non pregnant controls After delivery, T_m is normalized before S

Galactose tolerance test

Urinary galactose excretion after an oral 40 g galactose load was found to be normal in 20 pregnancies at term by Nurnberger while others claim it to be 'often abnormal (Friedberg 1960)

Serum alkaline phosphatase

Mean alkaline phosphatase activity rises slightly during the first half of pregnancy and then sharply during the seventh month, reaching a peak at term (Bodansky et al Hoch et al Speert et al Mean values obtained by different methods are given in Table 1 With different techniques the trend is the same but mean activities during the tenth

In the detailed and serial observation of 21 women during their whole pregnancy by Coryell et al total serum proteins declined by 13 % towards term. The albumin/globulin ratio fell from 1 32 before pregnancy to 1 21 in the second trimester, to 0 84 in the third trimester and to a low of 0 7 a few days after delivery On electrophoresis there was a fall in albumin and gamma globulin and a rise in the alpha 1, alpha 2 and beta globulin fractions as well as in fibrinogen These changes disappeared 6 to 12 weeks following delivery

The erythrocyte sedimentation rate increases from the sixth month until term to about 30 mm per hour, Wintrobe (Tysoe and Lowenstein) This is probably related to the increase in fibrinogen

Serum turbidity and flocculation tests

The reports regarding incidence of positive turbidity or flocculation tests during pregnancy are conflicting

The *thymol turbidity* was normal in all of Thorling s 201 patients and in the 27 patients of Christhilf and Bonsnes On the other hand Labo et al found 6 6 % abnormal reactions among 75 patients near term Friedberg et al 10 3 % among 120 patients Ikonen in 12 % among 100 patients McNair and Jaynes 6 % in early and 15 % in late pregnancy among some 300 patients and Dieckmann et al 15 % positives in his 85 patients throughout pregnancy

The *Takata reaction* was normal in all of Thorling s 197 cases and positive in 20 % of Friedberg s 120 patients, The latter also found 20 % positive *cadmium reactions*

The *cephalin flocculation test* was normal in all of Thorling s 197 and Christhilf and Bonsnes 27 patients Salmon found only 1 positive reaction in 71 patients In contrast to these studies Labo et al observed 6 6 % positive tests among 75 patients, McNair and Jaynes 8 % among 300 at term and Day and Hellestrand even 22 5 % of 101 patients

It is possible that the test results are highly influenced by the specific technique and test reagents used in the different laboratories Dieckmann et al for instance reported in 1951 around 20 % positive cephalin flocculation tests among 85 women and even 40 % positives in the first trimester but 3 years later Dieckmann and Pottinger found only 5 2 % pathological reactions in 225 patients

Conclusions

Of the many procedures used to evaluate liver function' only the serum transaminases and the prothrombin time remain within normal limits in all pregnant women Liver blood flow remains quantitatively unchanged while cardiac output increases Liver histology may show minor and nonspecific changes, but is generally considered to be within normal limits

There is an increased incidence towards term in spider naevi and palmar erythema The following laboratory test results are often increased progressively with advancing pregnancy total white cell count, number of segmented and non segmented neutrophils myelocytes and metamyelocytes serum alkaline phosphatase serum cholesterol, total serum lipids bromsulfalein retention,

al 280 cases) Among Ikonen's 99 cases 96 were normal and 3 had SGOT levels between 40 and 80 units, all 3 being patients in labor Mean SGOT levels during pregnancy are generally lower than in nonpregnant controls Among West and Zimmerman's 70 cases none surpassed a value of 15 units during the first two trimesters The means tend to rise toward term but remain within normal limits

Serum glutamic pyruvic transaminase behaves identically (Rimbach and Bonow 25 cases, Ikonen 99 cases) Occasional elevations up to 80 units may be observed during labor

Reports on *lactic dehydrogenase* during pregnancy are more conflicting Normal values in all patients were reported by Linton and Miller (33 cases), Knutson et al (100 cases), and Kubli (44 cases) West and Zimmerman report an increase in 2 out of 70 cases, but Hill in 19 out of 40 and Stone et al in 9 out of 71 cases During labor lactic dehydrogenase is elevated in nearly half the cases (West and Zimmerman 11 of 27, Kubli 13 of 35)

Serum ornithyl carbamyl transferase was slightly elevated in 7 of 16 normal pregnancies (Reichard et al)

Serum cholinesterase activity diminishes progressively throughout pregnancy (Friedman et al) Abnormally low values (less than 170 units) were found in 17 % of patients during the first trimester, in 33 % during the second trimester and in 43 % during the third trimester (Wetstone et al)

Serum tributyrinase also shows a slight decrease in mean activity towards term (Friedman et al)

Serum cholesterol and serum lipids

Serum cholesterol levels begin to rise in about the 4th pregnancy month and usually reach a maximum in the 8th month McNair and Jaynes found values over 250 mg per 100 ml in 9 % of normal pregnancies in the 4th month, in 53 % in the 6th month and in 57 % in the 8th month Similar findings were obtained by Wetstone et al In the third trimester 6 of their 11 patients had serum cholesterol levels over 300 mg per 100 ml with a maximum of 435 mg per 100 ml Serum cholesterol varied from 250 to 510 mg per 100 ml in 100 females with uncomplicated pregnancy near term reported by Ikonen, with the majority in the range of 300 to 420 mg per 100 ml Von Studnitz, in a careful study of 101 females during the course of their pregnancy, found also an increase in total lipids, alpha lipoproteins, beta lipoproteins and phospholipids These changes are presumed to be due to hormonal influence

Total serum proteins and serum electrophoresis

Total serum protein levels fall gradually during pregnancy, reaching a low at term (Pfau, Wetstone et al McNair and Jaynes) In a series of over 300 pregnant women McNair and Jaynes found serum protein levels below 6 gm per 100 ml in 7 % of patients during the 2nd pregnancy month and in 43 % during the 8th month It must be remembered that during pregnancy circulating plasma volume increases by about 50—65 % and total body water by about 20 %

In the detailed and serial observation of 21 women during their whole pregnancy by Coryell et al *total serum proteins declined by 13 % towards term.* The albumin/globulin ratio fell from 1 32 *before pregnancy to* 1 21 in the *second trimester,* to 0 84 in the third trimester and to a low of 0 7 a few days after delivery On electrophoresis there was a fall in albumin and gamma globulin and a rise in the alpha 1, alpha 2 and beta globulin fractions as well as in fibrinogen These changes disappeared 6 to 12 weeks following delivery

The erythrocyte sedimentation rate increases from the sixth month until term to about 30 mm per hour, Wintrobe (Tysoe and Lowenstein) This is probably related to the increase in fibrinogen

Serum turbidity and flocculation tests

The reports regarding incidence of positive turbidity or flocculation tests during pregnancy are conflicting

The *thymol turbidity* was normal in all of Thorling s 201 patients and in the 27 patients of Christhilf and Bonsnes On the other hand Labo et al found 66 % abnormal reactions among 75 patients near term Friedberg et al 10 3 % *among* 120 *patients* Ikonen in 12 % among 100 patients McNair and Jaynes 6 % in early and 15 % in late pregnancy among some 300 patients and Dieckmann et al 15 % positives in his 85 patients throughout pregnancy

The *Takata reaction* war normal in all of Thorling s 197 cases and positive in 20 % of Friedberg s 120 patients The latter also found 20 % positive *cadmium reactions*

The *cephalin flocculation test* was normal in all of Thorling's 197 and Christhilf and Bonsnes 27 patients Salmon found only 1 positive reaction in 71 patients In contrast to these studies Labo et al observed 66 % positive tests among 75 patients, McNair and Jaynes 8 % among 300 at term and Day and Hellestrand even 22 5 % of 101 patients

It is possible that the test results are highly influenced by the specific technique and test reagents used in the different laboratories Dieckmann et al for instance reported in 1951 around 20 % positive cephalin flocculation tests among 85 women and *even 40 % positives in the* first trimester but 3 years later Dieckmann and *Pottinger found only 5 2 % pathological reactions in 225 patients.*

Conclusions

Of the many procedures used to evaluate 'liver function' only the serum transaminases and the prothrombin time remain within normal limits in all pregnant women Liver blood flow remains quantitatively unchanged while cardiac output increases Liver histology may show minor and nonspecific changes but is generally considered to be within normal limits

There is an increased incidence towards term in spider naevi and palmar erythema The following laboratory test results are often increased progressively with advancing pregnancy total white cell count, number of segmented and non segmented neutrophils myelocytes and metamyelocytes serum alkaline phosphatase serum cholesterol total serum lipids bromsulfalein retention,

hepatic bromsulfalein storage, serum alpha and beta globulins, fibrinogen and erythrocyte sedimentation rate

An occasional increase not dependant on the stage of gestation is seen in serum bilirubin (up to 2 mg per 100 ml) and in the incidence of pathological serum turbidity and flocculation reactions No careful studies exist on the incidence of pathological bile components in the urine It is suggested that occasionally bilirubin, urobilin or increased amounts of urobilinogen may be present in uncomplicated pregnancy

There is a decrease towards term in hemoglobin, erythrocyte count, hematocrit, total serum proteins, serum bumin, gamma globulin, serum ch esterase, intravenous bilirubin toler and maximal bromsulfalein excretory pacity (T_m)

With the exception of total white counts most deviations from the are minor The main diagnostic di culties will be encountered with eleva alkaline phosphatase levels and path logical turbidity and flocculation te Determination of serum is the single most valuable test dur pregnancy, although a normal does not exclude the presence of live disease

II Jaundice during pregnancy

1) Incidence of jaundice during pregnancy

Jaundice during pregnancy is rare. Table 2 is an attempt to summarize all reports in the literature from which incidence can be calculated. The overall figure is 557 cases of jaundice in 822 842 pregnancies, an incidence of 0 067 % or cases per 10 000 pregnancies or 1 case per 1 500 pregnancies. The total series has been subdivided in 2 parts: 10 reports which give hepatitis as only diagnosis and 11 reports which list different etiologies including every case of jaundice during pregnancy. As can be seen, incidence of jaundice is slightly higher in the series which list hepatitis only. This does not make much sense. It may indicate that total incidence of jaundice during pregnancy reflects mainly the frequency of hepatitis during pregnancy in the respective series or that reports are mainly written after the experience of a hepatitis epidemic. Hepatitis accounts for at least 41 % of all cases with jaundice during pregnancy (Table 4). This figure may in some area during certain time periods approach 100.

In the single reports incidence of jaundice during pregnancy varies between 2 per 10 000 and 3 per 1 000 pregnancies, with the exception of 3 series collected during the height of a hepatitis epidemic. The highest observed incidence in reports covering more than a one year period is 8 hepatitis cases per 1 000 pregnancies (*Zondek and Bromberg*).

Excluding again series with observation periods of one year or less, the incidence of jaundice per year varies between 0 3 and 10 6 cases, with an average of 3 cases per year (415 cases in 138 years).

2) Classification of jaundice during pregnancy

The oldest classification of jaundice during pregnancy into icterus levis (those who survive) and icterus gravis (those who die) originates probably from Frerichs (1858) and is still used in the French literature with the addition of a third category, ictere pseudo-grave (those who look like they are going to die but eventually survive). Such a grouping is certainly clear cut but helpful to the clinician only in retrospect.

More meaningful is an attempt at an etiologic classification separating jaundice caused by pregnancy per se (only seen in pregnant women) from jaundice occurring by chance during the course of an otherwise uncomplicated gestation (seen also in non pregnant females and in males). The classification given in

hepatic bromsulfalein storage, serum alpha and beta globulins, fibrinogen and erythrocyte sedimentation rate

An occasional increase not dependant on the stage of gestation is seen in serum bilirubin (up to 2 mg per 100 ml) and in the incidence of pathological serum turbidity and flocculation reactions No careful studies exist on the incidence of pathological bile components in the urine It is suggested that occasionally bilirubin, urobilin or increased amounts of urobilinogen may be present in uncomplicated pregnancy

There is a decrease towards term in hemoglobin, erythrocyte count, hematocrit, total serum proteins, serum albumin, gamma globulin, serum cholinesterase, intravenous bilirubin tolerance and maximal bromsulfalein excretory capacity (T_m)

With the exception of total white cell counts most deviations from the normal are minor The main diagnostic difficulties will be encountered with elevated alkaline phosphatase levels and pathological turbidity and flocculation tests Determination of serum transaminases is the single most valuable test during pregnancy, although a normal result does not exclude the presence of liver disease

II Jaundice during pregnancy

1) Incidence of jaundice during pregnancy

Jaundice during pregnancy is rare Table 2 is an attempt to summarize all reports in the literature from which incidence can be calculated The overall figure is 557 cases of jaundice in 822,842 pregnancies an incidence of 0 067 % or 7 cases per 10 000 pregnancies or 1 case per 1 500 pregnancies. The total series has been subdivided in 2 parts 10 reports which give hepatitis as only diagnosis and 11 reports which list different etiologies including every case of jaundice during pregnancy As can be seen incidence of jaundice is slightly higher in the series which list hepatitis only This does not make much sense It may indicate that total incidence of jaundice during pregnancy reflects mainly the frequency of hepatitis during pregnancy in the respective series or that reports are mainly written after the experience of a hepatitis epidemic Hepatitis accounts for at least 41 % of all cases with jaundice during pregnancy (see Table 4) This figure may in some areas during certain time periods approach 100 %

In the single reports incidence of jaundice during pregnancy varies between 2 per 10 000 and 3 per 1 000 pregnancies with the exception of 3 series collected during the height of a hepatitis epidemic The highest observed incidence in reports covering more than a one year period is 8 hepatitis cases per 1 000 pregnancies (Zondek and Bromberg)

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TABLE 2 Incidence of jaundice during pregnancy

Year	Authors	Country	Time period (years)	Total pregnancies	Patients with jaundice	Incidence of jaundice: in % of all pregn	Incidence of jaundice: cases per year
Series with "hepatitis" as only diagnosis							
1943	Saurer	Zurich	1	2 764	3	0 108	–
1947	Zondek & Bromberg	Jerusalem	9	12 360	3	0 024	0 3
	Zondek & Bromberg	Jerusalem	2 6	3 382	27*	0 798	10 1
1950	Dill	Washington D C	3	25 000	12	0 048	4 0
1951	Mickal	New Orleans	9	69 186	15	0 022	1 7
1953	Martini et al	Hamburg	3 5	91 735	37	0 040	10 6
1955	Paul	Toronto	15	46 000	10	0 022	0 7
1956	Phatak & Patil	India	0 3	959	29	3 024	–
	Phatak & Patil	India	0 3	1 370	6	0 438	–
1957	Lacomme	Paris	?	10 000	10	0 100	–
1959	Mazaud et al	Dakar	3	3 969	10	0 252	3 3
1959	Peretz et al	Haifa	8	21 000	65	0 310	8 1
	Combined			287 725	227	0 078	
			53+		179+		3 4
Series with jaundice of different etiology							
1951	Javert & Morrison	New York	?	74 087	51	0 069	–
1955	Thorling	Uppsala	10	25 797	72	0 279	7 2
1955	Meyer	Halle	1	1 550	18	1 161	–
1957	Vincent	New Orleans	17	136 179	32	0 023	1 9
1957	Enrile et al	Philippines	7	14 944	7**	0 047	1 0
1961	Samuels	New Orleans	10	20 000	8	0 040	0 8
1962	Cahill	New York	28	110 378	52	0 047	1 8
1962	Synodinos et al	Athens	6	44 000	37	0 084	6 2
1962	Cremona	Torino	2	9 870	6	0 061	3 0
1963	Siegler & Keyser	New York	(7 10)	80 356	25	0 031	–
1966	Haemmerli & Wyss	Zurich	5	17 956	22	0 122	4 4
	Combined			535 117	330	0 061	
			85+		236+		2 8
	Both collective series combined			822 842	557	0 06[illegible]	
			138+		415+		3 0

* 29 cases in report 2 with anicteric hepatitis have been excluded

** 8 cases in report 1 case of acute cholecystitis without jaundice is excluded

\+ Excluding series with collection periods of 1 year or less

TABLE 3 Jaundice *during* pregnancy
(Synonym icterus gravidarum)

A. Jaundice *in* pregnancy
(*Synonyms icterus in graviditate* concommitant jaundice, coincidental jaundice, ictere intercurrent)

I Usual forms of jaundice occurring also in non pregnant subjects
1 Hepatic parenchymal disease (especially viral hepatitis)
2 Intrahepatic cholestasis (i e. drug jaundice)
3 Extrahepatic cholestasis (i e common duct stones)
4 Congenital *idiopathic* hyperbilirubinemias
5 Hemolytic disorders

II Jaundice in typical medical complications of pregnancy
1 Jaundice *in* severe pyelonephritis
2 Jaundice in pyelonephritis and tetracycline toxicity
3 *Delayed chloroform poisoning*
4 Jaundice after (criminal) abortions
(Clostridium perfringens septicemia, quinine toxicity etc)

B Jaundice *of* pregnancy
(Synonyms icterus e graviditate icterus graviditatis, icterus peculiar to pregnancy ictere lie à la grossesse)

I *Idiopathic jaundice of pregnancy*
1 Intrahepatic cholestasis of pregnancy
(jaundice of late pregnancy recurrent jaundice of pregnancy)
2 Acute fatty metamorphosis of pregnancy
(obstetric acute yellow atrophy)

II *Jaundice as a complication of another disease linked to pregnancy*
1 Jaundice in hyperemesis gravidarum
2 Jaundice in vomiting of late pregnancy
3 Jaundice in severe toxemia *of* pregnancy
4 Jaundice in megaloblastic anemia of pregnancy
5 *Jaundice in hemolytic anemia of pregnancy*

Table 3 seems to represent best our present state of knowledge

Reviews of jaundice during pregnancy have been published by Mayer 1906 Kehrer 1907 Schickele 1910 Seitz 1916 Rissmann 1917 Eppinger 1923 Holmer 1927 Chabrol 1932 Eppinger 1937 Verhage 1940 Seitz 1948 Puyo 1953 Lichtman 1953 Dietel 1954 Caroli et al 1954 Thorling 1955 Lacomme 1957 Bret and Seneze 1957 and 1958 Wilken 1958 Cattan and Cattan 1959 Dominici 1960 Richman 1960 Friedberg 1960 Labby 1960 Sheehan 1961, Meeroff 1961 Boquien et al 1961, Cremona and Voghera 1962 Cahill 1962 Synodinos et al 1962 Synodinos 1963, Friedberg 1963 Sherlock 1963, Ikonen 1964 and Gerl and Bonow 1964 The following reviews fail to mention recurrent jaundice of pregnancy (intrahepatic cholestasis of pregnancy) Winter 1890

TABLE 2 Incidence of jaundice during pregnancy

Year	Authors	Country	Time period (years)	Total pregnancies	Patients with jaundice	Incidence of jaundice: in % of all pregn	Incidence of jaundice: cases per year
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1951	Mickal	New Orleans	9	69,186	15	0 022	1 7
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1955	Paul	Toronto	15	46 000	10	0 022	0 7
1956	Phatak & Patil	India	0 3	959	29	3 024	—
	Phatak & Patil	India	0 3	1,370	6	0 438	—
1957	Lacomme	Paris	?	10 000	10	0 100	—
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	Combined			287,725	227	0 078	
			53+		179+		3 4
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1966	Haemmerli & Wyss	Zurich	5	17 956	22	0 122	4 4
	Combined			535 117	330	0 061	
			85+		236+		2 8
	Both collective series combined			822 842	557	0 067	
			138+		415+		3 0

* 29 cases in report 2 with anicteric hepatitis have been excluded

** 8 cases in report 1 case of acute cholecystitis without jaundice is excluded

\+ Excluding series with collection periods of 1 year or less.

TABLE 5 Jaundice during pregnancy. Frequency distribution of different diseases in 15 series combined (456 cases)

Diagnosis	Number of cases			%
Jaundice in pregnancy				
1 Hepatocellular jaundice				
Infectious hepatitis	189			
Acute yellow atrophy non-specified	7			
Cirrhosis	4			
Leptospirosis (Weil's disease)	1			
Liver metastasis	1	212		
Tuberculoma of liver	1			
Echinococcus of liver	2			
Chlorpromazine jaundice	6			
P.A.S. jaundice	1			
2 Extrahepatic obstruction				
Common duct stones	27	28		
Adenocarcinoma of common duct	1			
3 Familial non hemolytic jaundice		2	268	58.8
4 Hemolytic jaundice				
Sickle cell anemia	8			
After blood transfusion	4			
Hemoglobin H disease	1	19		
Familial hemolysis (Spherocytosis)	2			
Not specified	4			
5 Mixed forms				
Leukemia	2			
Appendicitis with perforation	1			
Infected cystadenoma of ovary	1	6		
Septic shock	2			
Jaundice due to complications of pregnancy				
Hyperemesis gravidarum	27			
Eclampsia	21			
Marked hypertension without eclampsia	3		60	13.9
Severe pre-eclampsia with jaundice	5			
After Hydatiform [illegible]	1			
After criminal abortion	3			
Jaundice of pregnancy				
Intrahepatic cholestasis of pregnancy	65			
Recurrent intrahepatic cholestasis of pregnancy	29		96 =	21.0
Acute fatty metamorphosis of pregnancy	2			
No diagnosis			33 =	7.2
			456 =	100.0

References for table 5: Jagert & Morrison; Ennle et al.; Vincent; Synodinos et al.; Cal [illegible]; Cre sona; Samuels; Siegler & Keyser; Ikonen; Verhage; Thorling; Barry & O'Dwyer; Nixon et al. References for unnumbered diagnosis are given in table 4.

TABLE 4 Jaundice during pregnancy Frequency distribution of different diseases in single reports

Year	Author	Total cases with jaundice	Hepatitis	Common duct stones	Hemolysis	Hyperemesis	Eclampsia	Cholestasis of pregnancy	Recurrent cholestasis of pregnancy	Other*	No diagnosis
1940	Verhage	43	6	2	1	15	14	—	1	4	—
1947	Nixon et al	12	9	—	—	—	1	—	—	1	1
1951	Javert & Morrison	51	20	9	6	3	—	—	—	13	—
1955	Thorling	72	24	—	—	6	—	28	3	7	4
1955	Barry & O Dwyer	9	5	—	—	—	1	1	—	2	—
1955	Meyer	18	8	1	1	3	3	—	2	—	—
1957	Vincent	32	25	2	—	—	—	—	—	5	—
1957	Enrile et al	7	3	1	—	—	1	—	—	2	—
1961	Samuels	8	6	1	—	—	—	—	—	1	—
1962	Cahill	52	29	3	—	—	1	—	4	1	14
1962	Synodinos et al.	37	27	—	9	—	—	—	—	1	—
1962	Cremona	6	4	—	—	—	—	—	—	2	—
1963	Siegler & Keyser	25	12	2	1	—	—	1	—	5	4
1964	Ikonen	62	5	5	1	—	—	35	13	2	1
1966	Haemmerli & Wyss	22	6	1	—	—	—	—	6	—	9
	Combined series	456	189	27	19	27	21	65	29	46	33
	in %	100	41 5	5 9	4 2	5 9	4 6	14 2	6 4	10 1	7 2

* Detailed in table 5

von Winckel 1893, Vinay 1894, Quirino et al 1948, Lock et al 1953, Puder 1955, Williams 1957, Millen 1957, Imparato 1958 and Varangot 1962 By far the most outstanding attempt at a clinical differential diagnosis is provided in Thorling s monography (1955)

3) Frequency distribution of different diseases causing jaundice during pregnancy

Fifteen reports including 11 used in Table 2, break down their cases of jaundice during pregnancy into different etiologic categories The 456 cases are summarized in tables 4 and 5 It is clearly evident that infectious hepatitis constitutes the most frequent single disease entity The figure of 41 5 % would be much higher, if the reports listing hepatitis as only diagnosis had been included Many of these hepatitis reports exclude just a few cases of other etiologies while some authors seem to feel that every case of jaundice during pregnancy is caused by viral hepatitis

Table 4 lists the etiologic distribution reported by the individual authors

TABLE 6 Incidence of viral hepatitis in relation to stage of gestation

Year	Authors	Cases of hepatitis Total	1 Trim	2 Trim	3 Trim
1951	Ingerslev & Teilum	88	2	1	85
1951	Javert & Morrison	20	5	2	13
1951	Mickal	15	0	4	11
1954	Ellegast et al.	52	14	21	17
1954	Frucht & Metcalfe	17	0	2	15
1955	Thorling	24	5	12	7
1955	Long Boysen & Priest	10	1	3	6
1955	Hartmann & Schoen	26	6	10	10
1956	Ezes & Bourdon	34	2	25	7
1957	Dorfler	55	10	25	20
1959	Peretz et al.	65	7	25	33
1961	Denning & Bruckmann	21	7	4	10
1962	Cahill	29	8	6	15
	Total	456	67	140	249
	in %	100	14.7	30.7	54.6

4) Review of literature on jaundice during pregnancy

The following chapters are an attempt to summarize our present state of knowledge concerning the different forms of jaundice during pregnancy by a review of the available world literature.

Infectious hepatitis during pregnancy

The elder literature and even some of the newer textbooks contain the following statements: pregnant women are especially susceptible to hepatitis; hepatitis in pregnant women occurs most frequently in the last trimester of pregnancy; hepatitis runs a severe course during pregnancy and results in a very high mortality. All these statements do not bear out under a critical evaluation of the literature.

Susceptability of pregnant women to viral hepatitis. The incidence of hepatitis in pregnant women runs parallel to epidemics in the general population. This is shown clearly in 5 reports. Zondeck and Bromberg in Jerusalem observed from 1934 to 1943 only 3 cases in 12 360 pregnancies, while in a 32 months period during 1943—1946 they saw 29 cases among 3,382 pregnancies. Peretz et al. reported 65 cases of hepatitis in Haifa during 1950 to 1957, 33 of which occured during 1950/1951. Ingerslev and Teilum in Copenhagen observed 15 cases during 1928 to 1940 and 91 cases during 1941 to 1949. Phatak and Patil in India reported in two successive 4 months periods 29 cases among

These figures are in all likelyhood not accurate They may reflect the trends in diagnosis by different clinicians and in different countries rather than actual incidence For example, 63 out of the 65 cases of non recurrent intrahepatic cholestasis of pregnancy are reported by two authors (Thorling and Ikonen) One case in our table is listed as 'hepatitis' in the original report, but has been otherwise classified by us on the basis of the detailed published data (case 1 in Barry and O'Dwyer) It is very likely that many cases of intrahepatic cholestasis of pregnancy are hidden under the diagnosis "hepatitis", especially in series where this is the only diagnosis made For instance 3 of 6 biopsies in Ingerslev s and Teilum's series of 91 "hepatitis" and 1 of 6 biopsies in the 65 'hepatitis" of Peretz et al showed 'normal liver tissue, which is frequently seen in intrahepatic cholestasis of pregnancy Intrahepatic cholestasis of pregnancy may possibly also be reported under jaundice due to choledocholithiasis, because the laboratory findings in both diseases may be identical It is interesting in this regard, that 9 out of 27 cases with jaundice ascribed to common duct stones are contained in one single report A rather interesting observation is that only 6 of the 15 reports admit to having cases of jaundice where no definite diagnosis could be reached

Trends inherent in the time period during which the report was written may also influence the etiologic frequency distribution Of course no case of intrahepatic cholestasis of pregnancy will be reported before 1955 the year of Thorling s description of the disease Cases of jaundice due to choledocholithiasis are few in single series after 1951, perhaps due to the more wide spread use of cholangiography More than half of the cases of jaundice second to hyperemesis or eclampsia are reported by a single author in 1940 (Verhage) It is possible that these disorders are better treated today and do less frequently progress to a jaundiced stage

Geographic pathology also influences frequency distribution Nine of 19 cases due to hemolytic jaundice are reported from Athens, due to the frequency of sickle cell anemia among the population admitted to that particular hospital The same holds true for jaundice due to echinococcus of the liver

Table 5 lists all diagnosis made in the 15 reports It may serve as a survey of the rarer types of jaundice occasionally encountered It is interesting to note that among the 19 cases with hemolytic jaundice there is a conspicuous absence of "idiopathic hemolytic anemia of pregnancy and of 'pernicious anemia of pregnancy', two diseases quoted in most textbooks

Few valid conclusions may be drawn from this type of survey The diagnosis of hepatitis is probably made too often and on inconclusive evidence but still viral hepatitis will account for the majority of cases with jaundice during pregnancy Rarer than generally thought are obstructive jaundice due to common duct stones and hemolytic jaundice due to the state of gestation Wrong diagnosis are probably made most often in cases of intrahepatic cholestasis of pregnancy unless when recurrent in successive pregnancies.

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	Total	456	67	140	249
	n o	100	14 7	30 7	54 6

4 Rev ew of l terature on jaund ce during pregnancy

The follo ng chapters are an attempt to su mari e our present state of kno l edge concern ng the different forms of jaund ce during pregnancy by a rev e of the a a lable world literature

Infe u l epat t s dur ng pregnancy

The older l eratu e and even so ne of t e ne er tex books conta the follo s, ta e e s pregnant women are espec all suscept ble to hepatitis hepa pregnant omen occurs most freq en l n t e la t tri nester of preg na c l epat s runs a se ere course du g p eg nanc and results n a ery h gh nortal t All these statements do ot bear out under a crit cal evaluat on of t e l e ature

Susceptab l ty of pregnant women to ral l epat tis The ncidence of hepa t t s n pregnant women runs parallel to ep demics n the general population. Tl s is shown clearly in 5 reports Zondeck and Bromberg in Jerusalem observed from 1934 to 1943 only 3 cases n 1? 360 pregnancies, while n a 32 months period during 1943—1946 they sa ?9 cases among 3 382 pregnancies Peretz et al reported 65 cases of hepa t s in Ha fa during 1950 to 1957 33 of which occured during 1950/1951 Ingerslev and Te lum in Copenhagen observed 15 cases during 1928 to 1940 and 91 cases during 1941 to 1949 Phatak and Pat l n India reported in two suc ces e 4 months periods ?9 cases among

These figures are in all likelyhood not *accurate*. They may reflect the trends in diagnosis by different clinicians and in different countries rather than actual incidence. For example, 63 out of the 65 cases of non-recurrent intrahepatic cholestasis of pregnancy are reported by two authors (Thorling and Ikonen). One case in our table is listed as "hepatitis" in the original report, but has been otherwise classified by us on the basis of the detailed published data (case 1 in Barry and O'Dwyer). It is very likely that many cases of intrahepatic cholestasis of pregnancy are hidden under the diagnosis "hepatitis", especially in series where this is the only diagnosis made. For instance 3 of 6 biopsies in Ingerslev's and Teilum's series of 91 "hepatitis" and 1 of 6 biopsies in the 65 "hepatitis" of Peretz et al. showed "normal" liver tissue, which is frequently seen in intrahepatic cholestasis of pregnancy. Intrahepatic cholestasis of pregnancy may possibly also be reported under jaundice due to choledocholithiasis, because the laboratory findings in both diseases may be identical. It is interesting in this regard, that 9 out of 27 cases with jaundice ascribed to common duct stones are contained in one single report. A rather interesting observation is that only 6 of the 15 reports admit to having cases of jaundice where no definite diagnosis could be reached.

Trends inherintent in the time period during which the report was written may also influence the etiologic frequency distribution. Of course no case of intrahepatic cholestasis of pregnancy will be reported before 1955, the year of Thorling's description of the disease. Cases of jaundice due to choledocholithiasis are few in single series after 1951, perhaps due to the more wide spread use of cholangiography. More than half of the cases of jaundice second to hyperemesis or eclampsia are reported by a single author in 1940 (Verhage). It is possible that these disorders are better treated today and do less frequently progress to a jaundiced stage.

Geographic pathology also influences frequency distribution. Nine of 19 cases due to hemolytic jaundice are reported from Athens, due to the frequency of sickle cell anemia among the population admitted to that particular hospital. The same holds true for jaundice due to echinococcus of the liver.

Table 5 lists all diagnosis made in the 15 reports. It may serve as a survey of the rarer types of jaundice occasionally encountered. It is interesting to note that among the 19 cases with hemolytic jaundice there is a conspicuous absence of "idiopathic hemolytic anemia of pregnancy" and of "pernicious anemia of pregnancy", two diseases quoted in most textbooks.

Few valid conclusions may be drawn from this type of survey. The diagnosis of "hepatitis" is probably made too often and on inconclusive evidence, but still viral hepatitis will account for the majority of cases with jaundice during pregnancy. Rarer than generally thought are obstructive jaundice due to common duct stones and hemolytic jaundice due to the state of gestation. Wrong diagnosis are probably made most often in cases of intrahepatic cholestasis of pregnancy unless when recurrent in successive pregnancies.

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The severe cases from the Mediterranean area and Asia are not quite uniform In the series of Ezes and Bourdon all 22 lethal cases were admitted in coma and died very rapidly 7 of them even before delivery Eleven delivered stillborn children and 4 premature living babies 3 of which died shortly thereafter Bilirubin levels in the mothers varied between 2 9 and 10 mg per 100 ml alkaline phosphatases between 11 and 17 Bodansky units and blood urea nitrogen between 16 and 31 mg per 100 ml Outstanding features were very high white blood cell counts (25 to 30 000) low blood sugars (between 14 and 40 mg per 100 ml in most) and very low prothrombin times (5 to 23 %) (Houel et al, same cases reported by different authors 3 years later) There was usually diffuse and massive bleeding from the gastrointestinal tract without obvious source at postmortem examination

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Mortality from hepatitis during pregnancy Table 7 gives the mortality figures for viral hepatitis during pregnancy collected from 38 reports in the literature The overall figure of 92 deaths among 887 cases or 10 4 % appears markedly higher than the mortality in the general population from the same disease A more meaningful interpretation of the data is achieved by dividing the reports geographically

In Europe, a mortality of 1 8 % among 449 cases is certainly comparable to the usual mortality figures from hepatitis in the general population Only one series shows a high mortality with 25 % or 2 out of 8 patients (Dietel) Here a selection of cases must play an important role, because Martini et al report from the same town and from the same time period a series with 57 cases and a mortality of only 1 7 %

In marked contrast to the low mortality in Europe is the high mortality in the Mediterranean area (24 1 %) and in Asia (50 %) The mortality is high in all series except the one from Istambul This cannot be due to chance An especially virulent virus in that area could be a possible explanation Peretz et al report that in Israel the new immigrants are not immunized to the middle East strain of hepatitis virus More likely, the high mortality reflects the general debility and undernourishment of the indigene population The 22 deaths from the series from Algiers occurred all in moslem women, who were brought to the hospital already in coma and died within hours to 4 days In the report by Mazaud et al from Dakar 3 of 6 African women died, but none of 4 European females Zondek and Bromberg state that among their 27 patients 18 were definitely undernourished, including the 9 cases with a severe course. Their 5 deaths occurred in a 5 months period during the height of a virulent epidemic

French authors often state that the combination of pregnancy and hepatitis has a very grave outlook This view is readily understandable on the basis of the 3 reported French series 29 of 54 patients died, a mortality of 53 7 % However, these 3 series originate from Dakar, Tunis and Algiers, and no report on this topic has ever been published from the French mainland

The series from the United States show an overall mortality of 7 7 %, but the single reports have either a very low or a very high mortality This reflects probably the different hospital types in that country, admitting different population groups In hospitals serving mainly people of the lowest income groups, undernourishment and general

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In the reports from Europe, hepatitis during pregnancy is said to run a course no different from that in nonpregnant females or in males In Martini et al's series bilirubin levels averaged 6 7 mg per 100 ml (range 0 7 to 24 mg per 100 ml) The only death occurred in a 38 year old woman with severe pyelonephritis Thorling's 24 cases were all benign Mean duration of hospitalisation was 24 days Three authors classify their cases according to the severity of the clinical course (see Table 8)

Fllegast et al compare the course in their 69 pregnant females with 360 nonpregnant hospitalised hepatitis cases Mild forms were seen in 66 7 % of pregnant and 14 4 % of nonpregnant subjects, severe cases in 8 7 % of pregnant and in 15 8 % of nonpregnant patients The 25 cases reported by Hartmann and Schoen were especially mild, with mean serum bilirubin levels of only 1 7 mg per 100 ml and an average hospitalisation of 19 days Ingerslev and Teilum note also a high proportion of mild forms among their 91 patients They further state that in 69 patients jaundiced up to the time of delivery the postpartum course was surprisingly uniform, in as much as jaundice cleared pretty regularly on the 11th post partum day regardless of the duration of jaundice before delivery This last feature is unusual for hepatitis during pregnancy and it is likely, that this series from Copenhagen includes many cases of intrahepatic cholestasis of pregnancy which was still unknown in 1951 This assumption is supported by the fact that of their 6 liver biopsies only 3 showed hepatitis and the other 3 were normal Furthermore, the thymol turbidity test was normal in the majority of cases

It is possible that other series may be heavily weighed in favour of mild forms of hepatitis during pregnancy by undiagnosed cases of intrahepatic cholestasis of pregnancy Still the conclusion appears valid, that the course of hepatitis — at least in Europe — is not influenced by pregnancy

Pregnancy itself is influenced by hepatitis only by a tendency to premature delivery Despite the often low prothrombin time in hepatitis uterine bleeding during delivery is very rarely marked, not even in patients in hepatic coma (Zondek and Bromberg) Ingerslev and Teilum note that 16 % of their 91 patients lost more than 500 ml blood during delivery, while 8 % appears to be the norm in their hospital However, no case bled excessively

Sequellae from hepatitis during pregnancy Follow up studies in women with hepatitis during pregnancy have been performed in 10 cases by Mickal in 26 by Hartmann and Schoen and in 57 by Martini et al No evidence of liver pathology was detected Five of Martini et al s patients had subjective liver symptoms" but were found to have completely normal liver functions

The only disagreeing report is that of Frucht and Metcalfe, who re examined 11 women and found only 2 free of liver disease' One can hardly concur with this conclusion on the basis of their data Five women had 'enlarged livers five had bilirubin levels of 1 0 to 1 8 mg per 100 ml and 4 had slightly increased globulin levels (in two of these deter

TABLE 9 Fate of infants born from mothers with viral hepatitis

Author	Total cases of hepatitis	Spontaneous abortions	Premature deliveries	Deaths of premature babies	Deaths in deliveries at term
Dietel	6	0	1	0	
Martini	54	2	11	0	
Ellegast et al.	47	0	16	4	2
Hartmann & Schoen	26	1	5	1	
Thorling	22	1	0	0	
Dorfler	55	3	10	1	
Denning & Bruckmann	19	1	2	0	
Total Europe	229	8	45	6	2
Zondek & Bromberg	29	0	7	0	
Pirinoli	37	1	16	9	
Synodinos et al	20	0	1	1	1
Total Mediterranean	86	1	24	10	1
Mickal	13	0	1	1	
Roth	16	2	0	0	1
Paul	10	0	5	3	
Cahill	29	2	3	1	
Total North America	68	4	9	5	1
Total combined series	383	13	78	21	4
in	100	3 4	20 4	5 5	1 0

mined during a new pregnancy) However bromsulfalein excretion and flocculation tests were normal in all Similarly Ley and Liebl examined 42 women 2 to 8 years after their pregnancy with hepatitis They *found minor chemical* abnormalities in this group (thymol turbidity 2 + in 30 %, Takata reaction slightly positive in 10 % and borderline bilirubin elevation in 2 cases) but there was no difference when compared to a similar follow up study in 69 cases with hepatitis outside pregnancy One case only had chemical evidence of probable mild liver disease Zondek and Bromberg are the only authors to report 2 cases of chronic hepatitis among their 23 survivors

Thus, incidence of residual liver disease after hepatitis during pregnancy *is certainly not more frequent than in* patients with hepatitis outside of pregnancy

Child survival from mothers with hepatitis during pregnancy Hepatitis during pregnancy induces a tendency toward abortion or premature delivery as does any other form of jaundice The mechanism is unknown There is no

correlation between premature delivery and duration of jaundice, serum bilirubin level or severity of clinical course (Ellegast 1954 a) Survival of the babies depends on their stage of maturity at birth and not on the mother s disease Table 9 summarizes the available data from the literature, excluding cases with therapeutic abortions

In the combined series of 383 cases 9 9 % of the babies died 3 4 % abortions, 5 5 % prematures and 1 0 % of babies delivered at term There were 20 4 % premature deliveries (19 6 % in the European and 27 9 % in the Mediterranean series) Of all prematures 13 6 % died in the European and 41 7 % in the Mediterranean collective series

Harnack and Martini report 20 women who had hepatitis 1 to 7 months before conception and were not jaundiced during pregnancy There were 2 abortions and 1 premature delivery in this series

A follow up study in children born of mothers with infectious hepatitis is reported by Ellegast et al (1954 c) Only 1 child was transiently jaundiced on the 7th day for 1 week, the others did not show any evidence of liver disease

Transplacentar infection with hepatitis virus and incidence of malformation in babies of mothers with hepatitis during pregnancy The problem of transplacentar infection of the embryo or baby with the hepatitis virus has been extensively discussed by several authors (Stokes et al , von Harnack and Martini Ellegast et al 1954 c, Mansell, Dorfler 1957 and Dorfler 1958) The evidence for its occurrence is very meager

Hepatitis in babies within their first two mon hs of life does occur, but their mothers are but exceptionally jaundiced during pregnancy On the other hand, practically none of the babies of mothers with clear-cut hepatitis during pregnancy have hepatitis Ellegast et al theorise that the babies are protected by the antibodies of their mothers, and that infection would only occur when viremia in the mother is present shortly before delivery, i e before the formation of antibodies

Dorfler in 1958 collected the available reports on the incidence of malformations Among 528 cases of hepatitis during pregnancy he found 19 malformations (reported in Harnack and Martini, Ellegast et al 1954 c, Bickenbach, Mansell) This incidence of 3 5 % is not significantly different from the normally expected malformation rate of 2 4 % Not included in Dorfler s review are 2 malformations reported by Ingerslev and Teilum and 1 case reported by Cahill, all 3 with hepatitis in the second half of pregnancy Most of these mothers were ill with hepatitis during the last trimester of gestation, which would not account for the malformations in their babies Kellog and Wesp observed a woman with hepatitis one month before conception who then delivered a monster A causal relationship is possible in this case

Thus transplacentar infection with hepatitis virus and malformation of babies has not been completely ruled out but — if it does occur — appears to be extremely rare.

Jaundice in liver cirrhosis during pregnancy

Cirrhosis of the liver and pregnancy is a very rare coincidence Most cases of cirrhosis in women are seen past the childbearing age (Burslem et al) In young women with cirrhosis fertilisation is frequently precluded by the high incidence of amenorrhoea oligomenorrhoea and non-ovulatory cycling (Labby)

At least 31 patients with cirrhosis of the liver and pregnancy are reported in the literature (Scaglione 1923 Kraul 1927 Hesseltine 1930 Tenney and King 1933, Ashton 1934 Lascano and Pereyra 1936 Javert and Morrison 1951 3 cases Burslem et al 1952 2 cases Puyo 1953 2 cases Mack et al 1953 2 cases Slater 1954 Bearn et al 1956 2 cases Abrams 1956 Ennle et al 1957 Adno 1957 Nabriski et al 1958 2 cases Labby 1960 Moore and Hughes 1960 3 cases, O Leary and Bepko 1962 Bennet et al 1963 Slaughter and Krantz 1963 2 cases) The age ranges from 23 to 42 years in these patients Five underwent 2 pregnancies after cirrhosis had been documented (Burslem et al case 2, Abrams Nabriski et al case 2 Moore and Hughes case 3 Slaughter and Krantz case 1)

Not all cases are reported with enough details to allow for an exact tabulation In at least 10 of the 31 patients there were no signs of deterioration of liver function during pregnancy (Kraul Tenney and King Burslem et al both cases Bearn et al both cases Adno Nabriski et al case 2 Moore and Hughes case 1 Slaughter and Krantz case 2) An 11th case had no symptoms during the course of gestation and massive but controllable uterine bleeding during delivery (Ashton) At least 7 of these 11 patients had jaundice, ascites and/or hematemesis prior to the reported asymptomatic gestation

One patient developed ascites during pregnancy (Abrams) and in another pre-existing ascites increased (Hesseltine) A total of 8 patients were jaundiced during pregnancy In 4 pre existing jaundice became more intense (Slater, Labby, Moore and Hughes cases 2 and 3) and in 3 jaundice developed during the last trimester of pregnancy In 2 of these jaundice persisted for several months post partum (Puyo) and the third patient died 5 days after delivery in hepatic coma (Ennle et al) In the 8th patient jaundice decreased towards term (Bennet et al)

Only 4 patients developed hematemesis during pregnancy In one it started in the 20th week of gestation could not be controlled and the patient died (Lascano and Pereyra) In a second case hematemesis occurred during the 5th month and was successfully stopped by performing a porto-caval shunt (O Leary and Bepko) The third case had hematemesis near term and ceased to bleed after a Caesarian section (Nabriski et al case 1) In the 4th case hematemesis started during labor and the patient exsanguinated (Scaglione)

There are a total of 4 deaths attributable to a complication of cirrhosis during pregnancy 2 are due to hematemesis (in 1923 and 1936), 1 due to hepatic coma and a fourth case report gives no details (Javert and Morrison) In addition there were 2 late deaths 1 six

months post partum from the sequellae of a splenectomy (Ashton) and 1 a year after delivery from liver failure (Slater)

Only 5 of the 36 children in this series died 2 still births in the 20th week (Lascano and Pereyra) and near term (Bennet et al), 1 of congenital abnormalities (Moore and Hughes case 3) and 2 during delivery (Mack et al case 2, Slaughter and Krantz case 1)

Five patients had *porto systemic shunts* performed prior to the reported pregnancy 2 porto-caval shunts (Bearn et al case 24, Abrams) and 3 splenorenal shunts (Adno, Labby, Slaughter and Krantz case 2) In all 5 there were no complications during gestation

In addition to these 31 patients with Laennec's or postnecrotic cirrhosis 3 pregnant patients with primary biliary cirrhosis are reported by Ahrens et al They suffered no ill effect from pregnancy

In general therefore, pregnancy is surprisingly well tolerated in a woman with cirrhosis of the liver It is probable that patients with the more severe forms of cirrhosis do not become pregnant and that the majority of cirrhotics with pregnancy represent a selection of benign cases

Drug-induced intrahepatic cholestasis during pregnancy

Of the long list of drugs capable of producing intrahepatic cholestasis (Dolle and Martini) *chlorpromazine* is the one most frequently used during pregnancy It has been advocated for the treatment of hyperemesis gravidarum by Moyer et al and by Benaron et al No cases of jaundice occurred in their 78 and 158 pregnancies respectively In the series of Stacey et al 8 of 170 non-pregnant patients (4 8 %) developed icterus Love and Peel collected 10 single case reports of chlorpromazine jaundice during pregnancy

Chlorpromazine jaundice during pregnancy, whether short lasting or chronic, has no ill effect on the child Clinically it may simulate intrahepatic cholestasis of pregnancy during the icteric stage It usually starts much earlier because the drug is given for hyperemesis during the first trimester and usually clears well before delivery Onset of chlorpromazine jaundice is within 4 weeks after the drug has been started There is a prodromal phase of 4 to 5 days' duration, usually acute with malaise, fever, chills, nausea mild abdominal pains, myalgias and occasionally skin rashes Itching may precede jaundice (Werther and Korelitz, Sherlock) These prodromal symptoms are similar to those seen in viral hepatitis and will readily distinguish drug-induced cholestasis from intrahepatic cholestasis of pregnancy, in which there are no prodromi except pruritus

On occasion chlorpromazine jaundice may become chronic, and the question whether pregnancy may induce chronicity is unsettled Read et al collected 22 cases with chlorpromazine jaundice of more than 3 months duration Three of these started during pregnancy and include the case with the longest duration on record, i e 3 years This woman (case 1 of Read et al) was given chlorpromazine 75 mg daily during 8 days for hyperemesis and developed intrahepatic cholestasis with a maximum serum bilirubin of 21 6 mg per 100 ml

an alkaline phosphatase of 256 King—Armstrong units and a serum cholesterol of 1,120 mg per 100 ml. Later xanthomatosis appeared and the clinical picture resembled primary biliary cirrhosis. The jaundice cleared after 3 years and the xanthomas were fading after 4 years. A case of 10 months duration is recorded by Gebhardt et al and one of 7 months duration by Stacey et al. A fourth case of chronic chlorpromazine jaundice starting during pregnancy (not contained in Read's et al's review) is reported by Love and Peel with a duration of 6 months. All patients recovered completely. The longest duration of icterus in non pregnant subjects has been 9 and 13 months, with jaundice of less than 7 months duration in all the others.

Drug induced intrahepatic cholestasis, clinically and histologically of the chlorpromazine type has also been described after *nitrofurantoin* (Furodantin®) in an elderly female (Ernaelsteen and Williams) and recently in a pregnant woman (Goldstein and Contino). In both instances pyelonephritis for which the drug was given was severe but responded rapidly to treatment, whereas jaundice persisted for some weeks.

Obstructive jaundice due to choledocholithiasis during pregnancy

Jaundice due to common duct obstruction by gallstones is rare during pregnancy. In our collective series on jaundice during pregnancy there are only 27 cases recorded (table 4). Nine of these are contained in a single paper which casts some doubt on the diagnostic accuracy. The rarity of this association even provokes single case reports (Rissmann 1909 and 1910, Brocq et al, Alex).

Gallstones have been said to occur more frequently in women with previous pregnancies than in childless women (Horn). Potter noted large atonic gall bladders with thick, tarry and viscous bile in three fourth of 390 women undergoing Caesarian sections. However, this did not cause stone formation. Large et al examined 352 pregnant women with cholecystographies and found gallstones in only 11. Furthermore, a large collective autopsy statistic by Robertson and Dochat showed that of 14,016 women with gallstones at autopsy 79.6 % had been previously pregnant but the incidence of previous pregnancies was nearly identical (79.2 %) in autopsies of females without gallstones.

Jaundice during pregnancy due to an *adenocarcinoma* of the common duct has been noted twice (Caroli et al 1954, Vincent).

Effect of pregnancy in chronic idiopathic hyperbilirubinemias (Dubin—Johnson syndrome Roter syndrome Gilbert—Meulengracht syndrome)

In a review in 1958 Dubin collected 53 cases of chronic idiopathic jaundice *(Dubin—Johnson syndrome)*. Nine of the 53 were women and 7 of the 9 underwent one or several *pregnancies*. (Dubin 1958 cases 16, 18 and 22, Klayman and Efrati, John and Knudtson, Tamaki, Carfagno). Pregnancy had no effect on the clinical course in one case, precipitated an attack of jaundice in 2

3—6535 6

months post partum from the sequellae of a splenectomy (Ashton) and 1 a year after delivery from liver failure (Slater)

Only 5 of the 36 children in this series died 2 still births in the 20th week (Lascano and Pereyra) and near term (Bennet et al), 1 of congenital abnormalities (Moore and Hughes case 3) and 2 during delivery (Mack et al case 2, Slaughter and Krantz case 1)

Five patients had porto systemic shunts performed prior to the reported pregnancy 2 porto-caval shunts (Bearn et al case 24, Abrams) and 3 spleno renal shunts (Adno, Labby, Slaughter and Krantz case 2) In all 5 there were no complications during gestation

In addition to these 31 patients with Laennec's or postnecrotic cirrhosis 3 pregnant patients with primary biliary cirrhosis are reported by Ahrens et al They suffered no ill effect from pregnancy

In general therefore, pregnancy is surprisingly well tolerated in a woman with cirrhosis of the liver It is probable that patients with the more severe forms of cirrhosis do not become pregnant and that the majority of cirrhotics with pregnancy represent a selection of benign cases

Drug induced intrahepatic cholestasis during pregnancy

Of the long list of drugs capable of producing intrahepatic cholestasis (Dolle and Martini) *chlorpromazine* is the one most frequently used during pregnancy It has been advocated for the treatment of hyperemesis gravidarum by Moyer et al and by Benaron et al No cases of jaundice occurred in their 78 and 158 pregnancies respectively In the series of Stacey et al 8 of 170 non pregnant patients (4 8 %) developed icterus Love and Peel collected 10 single case reports of chlorpromazine jaundice during pregnancy

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hemolytic jaundice will practically never pose a diagnostic problem

Rare causes of jaundice during pregnancy

Of course, any disease capable of producing jaundice may once in a while be encountered during pregnancy, such as echinococcus of the liver (Javert and Morrison, Synodinos et al), tuberculoma of the liver (Ennle et al) Weil s disease (Vincent), diffuse liver metastasis (Vincent) and leukemia (Javert and Morrison) A differential diagnosis of jaundice per se is beyond the scope of this paper

Jaundice in severe pyelonephritis during pregnancy

Before the advent of antibiotics pyelonephritis with jaundice was not infrequently seen during pregnancy (Decaudin) Jaundice was probably due to overwhelming septicemia. Toxic hemolysis may play an additional role (Rimbach)

Lepage in his thesis in 1934 collected 27 cases from the literature and added 4 of his own, calling the syndrome pyelonephrite dite gravido-toxique In our collected series from the literature since 1940 only 5 of 456 cases of jaundice during pregnancy were due to pyelonephritis (Table 5) Sheehan s autopsy series contains 12 pregnant women dying from pyelonephritis 3 of which had mild jaundice during the last few days of life The livers showed only mild non specific changes

This entity has now become rare Pyelonephritis can be treated today before it progresses to a jaundiced stage Effective treatment of pyelonephritis during pregnancy has however created a new hazard jaundice in pyelonephritis of pregnant women due to drug toxicity (see next paragraph)

Jaundice during pregnancy due to toxicity of drugs used in treatment of pyelonephritis (Tetracycline toxicity)

Schultz et al reported 6 autopsy cases of pregnant women treated for pyelonephritis with extremely high dosis of intravenous tetracycline (2 4 to 4 0 gm per day) The patients became jaundiced 3 to 5 days after treatment was started with bilirubin levels of 5 to 12 mg per 100 ml alkaline phosphatase of 9 to 44 King—Armstrong units, positive cephalin flocculations and mild elevation of serum glutamic oxalacetic transaminases (70—170 units) The patients died within 5 to 13 days The livers showed diffuse fatty metamorphosis

One of the two cases reported by Kahil et al and the case of Lewis et al, both diagnosed as acute fatty metamorphosis of pregnancy, had also received high dosis of tetracycline and streptomycin for severe pyelonephritis. At post mortem examination fatty metamorphosis was diffuse and the characteristic rim of intact liver cells in the periphery of all lobules seen in acute fatty metamorphosis of pregnancy was not present

Whalley et al reported 5 similar cases with survival in four Their treatment for pyelonephritis consisted of intravenous tetracycline 1.5—2 gm per day and intramuscular streptomycin 1 gm per day for 4—17 days All had renal failure and an associated pancreatitis Fatty metamorphosis was predominantly centrolobular in the liver biopsies of the 4

and aggravated pre-existing jaundice in 4. Icterus became gradually more intense, reached its height in the third trimester and waned or disappeared completely after delivery of the child. Clinically the disease may simulate extrahepatic biliary obstruction, but itching is absent and the alkaline phosphatase is usually within normal limits. Diagnosis may be suspected from the history of chronic jaundice without severe impairment of health and is easily verified by liver biopsy.

A similar syndrome but with normal liver biopsy findings has been described by Rotor. It is not clear at present, whether this is a disorder by itself or a milder form of the Dubin—Johnson syndrome. The patients with the *Rotor syndrome* have permanent mild jaundice but are otherwise asymptomatic. One woman reported in the literature underwent three pregnancies, each time with a decrease in the intensity of jaundice (Haverback and Wirtschafter, case 2).

In both the Rotor and the Dubin—Johnson syndrome the direct reacting serum bilirubin fraction is elevated. A normal direct reacting bilirubin with a mild elevation of the indirect-reacting fraction occurs in the *Gilbert—Meulengracht syndrome*. These patients are asymptomatic except for some fatigue (Foulk et al.). The syndrome occurs less frequently in females than in males. Stress, exercise and upper respiratory infections are said to increase jaundice temporarily. The effect of pregnancy on bilirubin levels in these patients has not been studied. Meulengracht reports two women who were free of jaundice during gestation.

Hemolytic jaundice during pregnancy. Hemolytic jaundice during pregnancy is very rare (Zachariae). It may be observed after incompatible blood transfusions. Jaundice observed in severe eclampsia and in overwhelming infections (pyelonephritis) has been classified by Sheehan as hemolytic.

Megaloblastic anemia of pregnancy may cause severe anemia, but rarely visible jaundice. In most cases it is due to dietary folic acid deficiency (Sanders). Occasionally vitamin B_{12} deficiency may be encountered, but is, if present, usually combined with folic acid deficiency (Lowenstein et al.). *Hemolytic anemia of pregnancy* (without demonstrable cause other than pregnancy itself) may produce jaundice, but is extremely rare (Dameshek and Schwartz, Crismer).

More often, hemolytic jaundice during pregnancy is caused by an exacerbation of a pre-existing chronic hemolytic state (Bromberg et al.). This may occur in congenital spherocytosis (Rimbach and Beckert) and in some hemoglobinopathies, especially in S—C, S—S and C—C disease (Fouche and Switzer, Curtis). Jaundice may become marked in some instances. The combination of gestation and hemoglobinopathy is hazardous for both mother and child.

A rare case may have a combined etiology such as megaloblastic anemia of pregnancy superimposed on congenital spherocytosis (Schneider and Frahm).

A classification of hemolytic anemias during pregnancy is given in the paper of Zachariae.

During pregnancy hemolysis presents as marked anemia and an accompanying

hemolytic jaundice will practically never pose a diagnostic problem

Rare causes of jaundice during pregnancy

Of course, any disease capable of producing jaundice may once in a while be encountered during pregnancy, such as echinococcus of the liver (Javert and Morrison Synodinos et al), tuberculoma of the liver (Ennle et al), Weil s disease (Vincent), diffuse liver metastasis (Vincent) and leukemia (Javert and Morrison) A differential diagnosis of jaundice per se is beyond the scope of this paper

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This observation implicates tetracycline as the responsible toxic agent. It is curious that this type of tetracycline toxicity has never been observed outside pregnancy and that the liver lesion is nearly indistinguishable from acute fatty metamorphosis of pregnancy, which does also not occur outside of gestation.

Nitrofurantoin (Furodantin®)-induced intrahepatic cholestasis is another but benign disorder occurring in the treatment of pyelonephritis. It occurs also in non pregnant subjects and has been discussed in the chapter on drug-induced cholestasis.

Jaundice due to delayed chloroform poisoning

This form of jaundice occurs only after delivery and is furthermore mainly of historic interest. It shall be mentioned here because it rarely occurred in non obstetric patients.

Sheehan reported 14 autopsy cases in 1940, which fell into 3 groups. Three patients apparently healthy aside from pregnancy had received an overdose of chloroform and died within 2 days without evidence of hepatic dysfunction. At autopsy there were isolated cell lesions involving more than half of the hepatocytes in the middle and center of the lobules. Nine patients with *prolonged* labor before chloroform narcosis developed jaundice, fever and coma on the second postpartum day and died 2 to 4 days later. The striking finding at autopsy was a band of mid zonal necrosis. Two patients with severe hyperemesis before chloroform narcosis had central necrosis at postmortem examination.

Intrahepatic cholestasis of pregnancy

Intrahepatic cholestasis of pregnancy is — after viral hepatitis — the second most frequent cause of jaundice during pregnancy. It accounts for 20.6 % of the 496 cases with jaundice listed in Table 4. It has a marked tendency to recur in subsequent pregnancies, but is entirely benign to both mother and child. The disease is characterized by marked pruritus followed by mild jaundice with no other prodromal symptoms and no impairment of general well being. Jaundice appears in the majority of cases after the 22nd week of gestation but may start as early as the 7th week in some patients. Jaundice and pruritus disappear rapidly after spontaneous or induced delivery. Biochemically the disorder presents the features of "obstructive jaundice" with no evidence of parenchymal liver cell damage. Bile flow in the extrahepatic biliary passages is unimpaired. Liver biopsies show minimal intrahepatic cholestasis.

Details on clinical, biochemical and

histological features of this disease will be discussed in the chapter on recurrent intrahepatic cholestasis of pregnancy

Acute fatty metamorphosis of pregnancy
In 1940 Sheehan delineated from true acute yellow atrophy of the liver due to fulminating viral hepatitis a new entity "obstetric acute yellow atrophy", which was later termed "acute fatty metamorphosis of pregnancy" by Ober and Lecompte Its etiology is unknown, but it occurs only in pregnant females This entity presents a clear cut and diagnostic histological picture of the liver, but is clinically nearly indistinguishable from fulminant viral hepatitis

Histologically it consists of a gross fatty change which starts in the center of the acini and involves finally the entire liver lobule with the exception of a sharply defined rim of normal liver cells around the portal tracts. There is a striking absence of necrosis and of inflammatory reaction Only occasionally a very light infiltration of small round cells may be seen throughout the fatty area The portal tracts show no abnormalities (Sheehan 1940) Bile thrombi may be present in the center of the lobules (Moore) Compared to fulminant hepatitis there is a striking lack of post mortem autolysis In the one survivor followed by serial liver biopsies (Whitacre and Fang) restitution of liver cells began from the remaining rim of normal cells in the periphery of the lobules and then progressed towards their center

Some confusion exists in the literature resulting from the inclusion of cases with diffuse fatty metamorphosis without a peripheral rim of intact liver cells Such cases probably do not belong to the entity described by Sheehan The case reported by Lewis et al for instance is a typical example of tetracycline toxicity in pyelonephritis during pregnancy Tetracycline toxicity must be implicated also in case 1 reported by Kahil et al

In the literature 40 cases of acute fatty metamorphosis of pregnancy have been reported (Stander and Cadden 1934, Cullinan 1936 cases A and B, Sheehan 1940 6 cases, Whitacre and Fang 1942 Nixon et al 1947 case 20 Barry and O Dwyer 1955 case 9, Ober and Lecompte 1955 3 cases, Moore 1955, Moore 1956 4 cases, Mason 1958, Dyson 1959, Nardone et al 1961, Sheehan 1961 2 cases Bruno and Ober 1962 5 cases Peters et al 1963 7 cases clinicopathologic conference 1963, Siegler and Keyser 1963 Kahil et al 1964 case 2) Not included in this review are 11 additional cases 6 because the histological findings are different or inconclusive (Baens and Espinola 1937 Dill 1950 cases 1 2 and 6, Lewis et al 1963, Kahil et al 1964 case 1) and 5 in which the patients recovered and no liver biopsies were performed (Duncan and McLachlan 1933 Taylor 1952 Pirrnoli 1957, Edwards 1960 Sheehan 1961)

Acute fatty metamorphosis of pregnancy has been noted in the age range of 16 (Ober and Lecompte case 3) to 42 years (Nardone et al, Dyson) In the majority of cases it occurred during the first pregnancy In only 3 of 40 cases it was observed after the second gestation during the third pregnancy in the CPC case during the sixth in Moore's case 2 and during the 8th in case 2 of Nardone et al.

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Details on clinical, biochemical and

al s cases Fatty degeneration of the renal tubules was noted in 7 cases on postmortem examination (Ober and Le compte 3 cases, *Dyson, Nardone et al*, Sheehan 1961, CPC) Associated pre-eclampsia was present in 5 patients (Nixon et al, Dyson, Sheehan 1940, Nardone et al, Kahil et al)

Of the 40 acceptable cases only 6 women survived Two of these are re ported in abstract form (Peters et al) and in a third no details are given (Siegler and Keyser) *Of the remaining* 3 cases 2 were delivered by an early Caesarian section (Whitacre and Fang, Moore case 2) and in one the first symptom occurred after delivery (Moore case 3) Another 5 cases with recovery but without histological proof of the diagnosis have been mentioned above

Only 5 children survived in the 40 acceptable cases one in a case with on set of symptoms after delivery (Nixon et al) the two children delivered by Caesarian section from which the moth ers survived also (Whitacre and Fang Moore case 2) and two after spontaneous delivery (Stander and Cadden Ober and Lecompte case 1)

Acute fatty metamorphosis of preg nancy is thus a rare but distinct entity not occurring outside of pregnancy It carries a grave prognostic outlook but is potentially reversible As rapid deteriora tion sets in always after delivery and as both women delivered by Caesarian *section survived it appears reasonable* to propose as an only chance for both mother and child a therapeutic Caesarian section as early as possible after onset of symptoms.

Jaundice in hyperemesis gravidarum

Jaundice in hyperemesis gravidarum is rare, usually mild and does not imply a *poor prognostic* outlook In a thesis in 1926 Ferru could collect only 22 cases from the literature, one of which died

Of 51 patients with hyperemesis re ported by Verhage 15 had a slight serum bilirubin elevation, but only a few were frankly jaundiced Urinary urobilinogen was increased in most and urine bili rubin was present in about one third. *Of Millars 120 patients 5 were jaun*diced In Klier's series of 119 patients 12 had elevated serum bilirubin levels ranging from 1 9 to 8 6 mg per 100 ml All had proteinuria and ketonuria The cadmium reaction was negative in all and the thymol turbidity positive in one A further 7 patients without jaundice had bilirubin and urobilin present in their urines All patients recovered Of Herold s 25 patients 8 had a serum bili-rubin of more than 1 mg per 100 ml, with 2 peak values of 4 4 and 6 7 mg per 100 ml Bilirubinuria was present in those with a serum bilirubin of more than 1.5 mg per 100 ml. Direct reacting bilirubin was positive in 19 and the galac-tose tolerance test impaired in 16 Thor-ling reports 6 patients with hyperemesis and jaundice Five had associated or pre ceding pruritus, which run parallel with the vomiting in three Three pa-tients had an elevated alkaline phos-phatase The thymol turbidity was nor-mal in all Both serum transaminases may be increased in the more severe cases SGOT up to 82 units SGPT up to 283 units (Durst and Strauss)

No liver biopsies have been performed in this disease Sheehan reported in

Onset of symptoms was in 30th week in two cases (Moore case 1 and 2), in the 31st week in 1 (Ober and Lecompte case 1), in the 34th in 2 (Moore case 4, CPC case) and in the others between the 36th and 40th week. The disease starts rather suddenly with severe and persistent vomiting, occasionally accompanied by abdominal pains, followed in a few days by jaundice. Tachycardia of between 100 and 130 is regularly present, but there is no fever. The symptoms then progress rapidly, the jaundice becomes intense, the patient somnolent, the vomiting assumes a coffee ground aspect and frank hematemesis may supervene. Severe headaches are noted in some patients. The laboratory features are consistent with an obstructive jaundice, except that urinary bilirubin may not be present until terminally. The highest recorded serum bilirubin levels were 12.7 (Whitacre and Fang), 16.8 (Moore case 3) and 26 mg (CPC case) per 100 ml, all others being below 10 mg per 100 ml. The alkaline phosphatase is increased to levels up to 54 King–Armstrong units (Moore). Serum transaminases have been recorded in only 2 cases with values between 100 and 300 units (CPC, Kahil et al.). Thymol turbidity and cephalin flocculation tests are normal with one exception (Kahil et al.). Prothrombin time is markedly decreased. White cell counts ranged from 19,200 to 32,000 per cu. mm in the 7 recorded cases (Barry and O'Dwyer, Ober and Lecompte, Kahil et al., CPC). A few patients had marked hypoglycemic episodes (Stander and Cadden, Whitacre and Fang, Kahil et al.). In about half of the cases oliguria sets in, followed by azotemia. A disproportionate rise of uric acid levels has been noted by Sheehan, and a disproportionate rise of serum creatinine in the CPC case. Others have seen a drop of the previously elevated blood urea nitrogen to abnormally low levels just before death (Barry and O'Dwyer, Mason, CPC).

The usual course is steadily downhill. Premature labor sets in, the woman delivers a stillborn child and then lapses into deep coma, occasionally accompanied by convulsions. High fevers may now occur and the patient dies shortly thereafter. The duration from the first symptom until death may vary between 3 days and 4 weeks and is usually between 1 and 2 weeks. The duration from delivery to death is usually 2 to 4 days, but may range from one hour (Ober and Lecompte case 3) to a maximum of 7 days (Moore case 4).

Some minor exceptions to this typical course may occur. Two cases died undelivered before ever having been jaundiced (Dyson, Nardone et al.), another died undelivered during the icteric stage (Cullinan, case A). In 2 others the first symptom appeared only after delivery (case 20 of Nixon et al., case 3 of Moore) and in one jaundice began 4 days post partum (Ober and Lecompte, case 2). In two cases pruritus was present (Ober and Lecompte case 1, CPC case). Four patients developed ascites (Ober and Lecompte case 1, Moore case 3, Whitacre and Fang, Kahil et al. case 2). Two patients bled profusely during delivery (Mason, CPC case). At autopsy a clinically unsuspected acute hemorrhagic pancreatitis was found in 3 of Bruno and Ober's and in 4 of Peters et

al s cases Fatty degeneration of the renal tubules was noted in 7 cases on postmortem examination (Ober and Le compte 3 cases, Dyson, Nardone et al, Sheehan 1961, CPC) Associated pre eclampsia was present in 5 patients (Nixon et al, Dyson, Sheehan 1940, Nardone et al, Kahil et al)

Of the 40 acceptable cases only 6 women survived Two of these are re ported in abstract form (Peters et al) and in a third no details are given (Siegler and Keyser) Of the remaining 3 cases 2 were delivered by an early Caesarian section (Whitacre and Fang, Moore case 2) and in one the first symp tom occurred after delivery (Moore case 3) Another 5 cases with recovery but without histological proof of the diagnosis have been mentioned above

Only 5 children survived in the 40 acceptable cases one in a case with on set of symptoms after delivery (Nixon et al) the two children delivered by Caesarian section from which the moth ers survived also (Whitacre and Fang Moore case 2) and two after spontaneous delivery (Stander and Cadden, Ober and Lecompte case 1)

Acute fatty metamorphosis of preg nancy is thus a rare but distinct entity not occurring outside of pregnancy It carries a grave prognostic outlook but is potentially reversible As rapid deteriora tion sets in always after delivery and as both women delivered by Caesarian section survived it appears reasonable to propose as an only chance for both mother and child a therapeutic Caesarian section as early as possible after onset of symptoms

Jaundice in hyperemesis gravidarum

Jaundice in hyperemesis gravidarum is rare, usually mild and does not imply a poor prognostic outlook In a thesis in 1926 Ferru could collect only 22 cases from the literature, one of which died

Of 51 patients with hyperemesis re- ported by Verhage 15 had a slight serum bilirubin elevation, but only a few were frankly jaundiced Urinary urobilinogen was increased in most and urine bili rubin was present in about one third Of Millar's 120 patients 5 were jaun diced In Klier's series of 119 patients 12 had elevated serum bilirubin levels ranging from 1 9 to 8 6 mg per 100 ml All had proteinuria and ketonuria The cadmium reaction was negative in all and the thymol turbidity positive in one A further 7 patients without jaundice had bilirubin and urobilin present in their urines All patients recovered Of Herold s 25 patients 8 had a serum bili rubin of more than 1 mg per 100 ml, with 2 peak values of 4 4 and 6 7 mg per 100 ml Bilirubinuria was present in those with a serum bilirubin of more than 1 5 mg per 100 ml Direct reacting bilirubin was positive in 19 and the galac tose tolerance test impaired in 16 Thor- ling reports 6 patients with hyperemesis and jaund ce Five had associated or pre ceding pruritus, which run parallel with the vomiting in three Three pa tients had an elevated alkaline phos- phatase The thymol turbidity was nor- mal in all Both serum transaminases may be increased in the more severe cases SGOT up to 82 units SGPT up to 283 units (Durst and Strauss)

No liver biopsies have been performed in this disease. Sheehan reported in

1939 19 autopsy cases of patients dying from hyperemesis Those with a duration of symptoms of more than 6 weeks were slightly jaundiced Thirteen had cerebral symptoms prior to death which were interpreted as Wernicke's encephalopathy Histologically the liver was normal in 7 and showed in 12 a slight fatty infiltration which was considered insignificant and did not lead to necrosis A specific liver lesion has not been found and most reported histological changes could also be due to starvation and avitaminosis

The incidence of hyperemesis gravidarum appears to be on the decline Guttmacher reports that in 1937 one in every 150 pregnant patients had to be hospitalised for hyperemesis, while in 1957 the the figure was only 1 in 974 In reviews on jaundice during pregnancy no series since 1940 reports more than 6 patients with jaundice due to hyperemesis (see Table 4) The clinical problem encountered will be mainly one of differential diagnosis from the prodromal phase of viral hepatitis, which should not be too difficult when tests such as serum transaminases and thymol turbidity are employed

Jaundice in hyperemesis may recur in successive pregnancies

Jaundice in vomiting of late pregnancy

Apart from hyperemesis gravidarum which occurs in the first trimester and almost always clears spontaneously by the 12th week of gestation there is a syndrome of "pernicious vomiting in late pregnancy" occurring usually between the 33rd and 37th week Almost always an underlying cause like pyelonephritis other infections or pre-eclampsia is found Sheehan reports an autopsy series of 16 such cases, of which 4 had a serum bilirubin elevation to maximally 2 mg per 100 ml in the last few days of life The livers showed histologically no significant changes or a slight fatty infiltration

Clinically this syndrome never presents as a case of jaundice The term should best be abandoned, because rarely does vomiting appear to be "idiopathic"

Jaundice in toxemia of pregnancy

Toxemia of pregnancy is accompanied by an increased incidence of abnormal so-called liver function tests, but liver function on the whole is not impaired Among 44 patients Dieckmann et al found in 51 % a positive cephalin flocculation and in 43 % a positive thymol turbidity test A slight increase in bromsulfalein retention was noted by Christhilf and Bonsnes Serum alkaline phosphatase was elevated in 75 % of Mukherjee's 100 toxemic patients, compared to a 28 % incidence in his normal pregnant controls The observed range was 12 5 to 16 5 King—Armstrong units in mild and 13 4 to 29 5 units in severe cases Alkaline phosphatase closely parallels the clinical course in eclampsia and increases with the number of convulsions Serum glutamic oxalacetic transaminase and lactic dehydrogenase are often elevated in the more severe cases (Kubli) Within the first 36 hours after admission SGOT was elevated in all of Crisp et al s 64 patients with values above 100 units (maximum 244 units) in all severe cases A rapid fall in SGOT indicated a good prognosis

Jaundice is rare in toxemia occurs

late in the course and often suggests a grave prognosis The bilirubin increase in 17 % of Dieckmann et al s 44 patients was only slight Of Verhage s 96 patients with toxemia only 11 had icterus, 2 of which died In Sheehan's *autopsy series* of 90 cases only 10 were jaundiced Sheehan believes the icterus to be due to hemolysis, as his jaundiced patients had hemoglobinuria during life and hemoglobin casts in the kidney tubules at autopsy

Histological liver lesions so striking at autopsy, are absent in mild and even in some far advanced cases on liver biopsies (Ingerslev and Teilum III) Of 15 severe cases with convulsions only 5 showed definite liver lesions in Antia et al s biopsy series In Sheehan s opinion histological changes occur only during the last two days of life

The liver lesions reflect the basic vascular disorder Characteristic findings consist of fibrin thrombi in the sinusoids of the periportal zone Similar thrombi occur in other organs. In the liver they obstruct sinusoidal blood flow and cause sinusoidal dilatation which leads to necrosis of clusters of hepatic cells (often wrongly called *fibrinoid necrosis*) and finally to hemorrhagic necrosis of the periportal zone If the patient has been *in shock centrolobular necrosis may be* superimposed and the areas of hemorrhagic necrosis may become confluent and form large blood pools An inflammatory reaction is characteristically absent Liver lesions in eclampsia are therefore interpreted as a terminal event (Sheehan Ingerslev and Teilum III Dietel 1947 Dieckmann 1952 Antia et al 1958)

The rare case of jaundice due to *toxemia of pregnancy* will hardly present clinically as an icterus of unknown origin

5) Indications for interruption of pregnancy *because of jaundice*

Interruption of pregnancy because of a disease causing jaundice during pregnancy is never indicated with the possible exception of acute fatty metamorphosis of pregnancy

This statement will not be questioned when applied to chronic idiopathic hyperbilirubinemias or to drug induced intrahepatic cholestasis and tetracycline toxicity In the rare case of jaundice due to common duct obstruction surgery on the biliary tract can be performed during gestation When jaundice of this kind develops after the 36th week surgery *may be delayed until after delivery* Treatment in jaundice due to pyelonephritis with septicemia is directed at the infection

Intrahepatic cholestasis of pregnancy is definitely not an indication to terminate pregnancy Although an induced *interruption of pregnancy will cure* the disorder such a cure will occur in all cases after spontaneous delivery Symptoms are never so distressing as to justify a shortening of the jaundiced period since pruritus can be effectively relieved by cholestyramine Many interruptions have been performed *especially* in the recurrent form, but usually by physicians who were not *familiar* with this disease and *its absolute* and constant benignity

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An interruption of pregnancy has

often been advocated in severe cases of viral hepatitis As has already been pointed out in the respective chapter, no evidence exists that the course of viral hepatitis is affected by pregnancy itself Pregnancy is influenced by viral hepatitis in a tendency to premature deliveries However, spontaneous abortions in severe cases of hepatitis do not improve the course of the disease, and furthermore a turn to the worse has been observed after spontaneous abortions In addition, any narcosis or surgery is very poorly tolerated in severe cases of hepatitis, which again is a strong point against a surgical termination of pregnancy

The course of a patient with liver cirrhosis is generally not influenced by pregnancy It has already been pointed out, that cirrhotic females with pregnancies present in a way a selection of cirrhotics with fairly good liver function, as in severe cases of cirrhosis fertilisation is rare Pregnancy may possibly increase portal hypertension by an increase in intraabdominal pressure In pregnant cirrhotics with hematemesis due to esophageal varices successful porto-caval shunts have been performed The rare case in which liver function decompensates critically during pregnancy *would probably have a poor prog*nostic outlook even without pregnancy Furthermore, in such a case surgery is as poorly tolerated as in severe viral hepatitis For all these reasons an induced termination of pregnancy appears not indicated in cirrhosis

In rare cases of severe hyperemesis gravidarum or severe toxemia of pregnancy a termination of gestation may become necessary The indications for an interruption of pregnancy will be based on general obstetrical principles and will not be based on the presence or absence of jaundice In hyperemesis gravidarum jaundice does not carry a grave prognosis In toxemia of pregnancy jaundice occurs only in the severe cases and its presence may be an additional *point for the obstetrician in fa*vour of a termination of gestation Valuable guides for the prognostic outlook in toxemia of pregnancy are the serum alkaline phosphatase and the serum transaminases

Acute fatty metamorphosis of pregnancy is probably a valuable and furthermore the only indication to terminate pregnancy because of jaundice In order to be successful a Caesarian section has to be performed very early in the course, which necessitates an early and correct diagnosis for which a liver biopsy is nearly indispensable As performance, embedding and reading of a liver biopsy usually takes two days in the most favorable circumstances, it may be necessary to avoid this delay and to take a decision without a biopsy, based on the typical clinical symptoms and signs

Interruption of pregnancy may be indicated in some hemoglobinopathies which are known to take a deleterious course under the influence of pregnancy (especially S—C, S—S and C—C disease) and in some hemolytic anemias during pregnancy not responding to medical treatment *In these instances the* decision will be taken because of hemolysis and not because of jaundice Jaundice if present is mild anyhow in these disorders

III Recurrent jaundice during pregnancy

Clear definitions of the different diseases disguised under the term recurrent jaundice during pregnancy are hampered by the similarity of the clinical course between many of these disorders, which are nearly all benign as evidenced by their recurrence during successive pregnancies Pujo wrote in his thesis in 1953 Les travaux publies, malgre le nombre de cas rapportes et le nombre de donnees (clinique biologique et histologique) souvent reuni sont mal utilisable quand on desire se faire une idee des particularites de ces icteres Furthermore the majority of case reports are but sparsely documented with laboratory data and rarely verified by liver biopsies The problem of delivering the pregnant patient usually takes precedence over the problem of investigating the cause of jaundice, especially as jaundice is mild in this syndrome and clears rapidly after delivery

1) Historical note

Recurrent jaundice during pregnancy has to our knowledge first been noted by Hoffman in 1872 (une jeune femme laquelle a chaque grossesse devenait icterique peu de temps avant d accoucher) The first case report giving some details was published by Ahlfeld in 1881 Up to 1910 15 cases were known in the medical literature During the next 12 years only 1 case was published Interest in this disease continued to be minor, with 11 cases reported between 1923 to 1935 and 5 cases reported from 1940 to 1948 The years 1950 to 1959 brought 30 new cases and the discovery of further patients promises to be profuse in the 1960 s

Many of the early authors concluded that in the case they described jaundice was in some way linked to the state of gestation With the description of additional cases it became apparent that at least two thirds of all patients with recurrent jaundice during pregnancy had similar and typical symptoms (defined on page 36) and that they therefore must represent a definite disease entity This disease, now called recurrent intrahepatic cholestasis of pregnancy, has been clearly and fully described by two Swedish authors, Svanborg in 1954 and Thorling in 1955 Both workers also defined the first attack, i e the non recurrent form of the same disease. The intrahepatic cholestasis mechanism has however been definitely expressed in a case report by Perreau in 1953 and was probably first alluded to by Boreel in 1924 at Van den Bergh s department The first liver biopsies in this disease were performed by Ljunggren in 1956 and Gros in 1958

The difficulties in clearly separating the different disorders causing recurrent

jaundice during pregnancy are best exemplified in the largest single series of cases (Perreau and Rouchy 1961). Of the 9 cases in the report only 7 conform to the diagnosis of recurrent *intrahepatic* cholestasis of pregnancy. Case II had histologically the features of cirrhosis of the liver and case III is an example of recurrent pruritus during pregnancy.

2) Recurrent intrahepatic cholestasis of pregnancy

Nomenclature

Recurrent jaundice of pregnancy has originally been used as a purely descriptive term, meaning the occurrence of jaundice during successive pregnancies. As more and more cases were described in the literature, the term assumed the meaning of a specific disease (Magnani 1924, Schwalm 1932, Perreau 1953). Finally, "recurrent jaundice of pregnancy" was used by Svanborg in 1954 to include also the non recurrent form of the same disease (i.e. first attack in just one pregnancy). While most cases described under this heading are examples of intrahepatic cholestasis of pregnancy, other forms of jaundice during pregnancy may also present as recurrent jaundice during successive gestations (discussed under differential diagnosis of recurrent jaundice), which further confuses the issue. In Thorling's opinion the term "recurrent jaundice of pregnancy" designating a form of jaundice peculiar to pregnancy, has become redundant.

We share Thorling's opinion and reject the term "recurrent jaundice *of* pregnancy" as meaning a specific disease, but we propose to retain the term "recurrent jaundice *during* pregnancy" as a purely descriptive one without etiological implications, just as the term "jaundice" alone is descriptive without implying a specific disease entity.

The disease formerly called "recurrent jaundice *of* pregnancy" is in our opinion best termed recurrent "intrahepatic cholestasis of pregnancy". A multitude of other names have been used for its designation. The term most frequently employed in the older literature is "icterus gravidarum" (Ahlfeld 1881, Becking 1896, Von den Velden 1904, Boreel 1924) or "Schwangerschaftsicterus" (Benedict 1902, Mayer 1906) and "Graviditatsicterus" (Brauer 1903). However, the same term served also to include all forms of jaundice during pregnancy. The word "idiopathic" was then added, as in "idiopathic hepatopathy of pregnancy" (Ljunggren 1956), "idiopathischer Schwangerschaftsikterus" (Eppinger 1937, Gros 1958, Wilken 1958) and "idiopathic jaundice of pregnancy" (McAllister and Waddell 1962, King and Kerrins 1963). The etiology of the disease is certainly unknown and the term "idiopathic" is at present justified, but "acute fatty metamorphosis of pregnancy" is also idiopathic and does also occur only in pregnant women (Moore 1963). "Benign jaundice of pregnancy" (Orellana et al. 1961) and "Hépatite bénigne de la grossesse" (Caroli et al.) appear correct but not precise enough.

Attempts at a pathogenetic description are expressed in the next group of terms: "Hepatosis" (Sheehan 1961) or "obstetric hepatosis" (Ikonen 1964) ex

Table 10 Recurrent jaundice during pregnancy Classification of cases used in review of literature

Category	Description		Number of cases	Tables
I	Recurrent intrahepatic cholestasis of pregnancy with liver biopsies		23	Table 11
	I A) with laboratory data and details on clinical course	18		Tables 13 16
	I B) without further details	5		Table 18
II	Probable recurrent intrahepatic cholestasis of pregnancy with sufficient laboratory data but without liver biopsies		20	Table 17
	II A) with details on clinical course	15		Table 14
	II B) without further details	5		Table 19
III	Recurrent jaundice during pregnancy with details on clinical course No biopsies no or scarce laboratory data		12	Table 15
IV	Recurrent jaundice during pregnancy number of gestations mentioned only		26	Table 20
V	Recurrent jaundice during pregnancy no details reported		23	Table 21
VI	Recurrent jaundice during pregnancy sufficient details reported to exclude intrahepatic cholestasis of pregnancy (*liver biopsy in 6 cases*)		28	—
Total cases of recurrent jaundice during pregnancy			132	
VII	*Non recurrent jaundice during pregnancy* considered to represent possibly intrahepatic cholestasis by original author or by reviewer		267	Table 22
	VII A) cases with liver biopsy	33		Table 12

References are given in the respective tables.

cludes an infectious agent but has been applied by others to jaundice seen in *hyperemesis and eclampsia* (Gros 1958)

Hepatite toxi gravidique (Caroli et al 1954) might as well be used in eclampsia with jaundice and is therefore non specific Endogenous hepatotoxemia of pregnancy (Thorling 1955) has the same disadvantage as idiopathic and could also *include acute fatty meta* morphosis of pregnancy Furthermore the term calls for a counterpart exogenous hepatotoxemia of pregnancy which was used by Thorling for jaundice during pyelonephritis in gestation but which might also be employed in other septicemias, in chloroform poisoning, or *in drug induced* jaundice

In a designation for the disease the intrahepatic cholestasis mechanism was first alluded to by Caroli et al (1954) in the term forme dite cholestatique pure des hepatites icterigenes de la grossesse later changed to ictere cholestatique de la grossesse by Perreau and Rouchy (1961) Intrahepatic cholestasis of pregnancy finally has been used by Haemmerli and Wyss for the presentation of their first 5 cases (Annual Meeting of the Swiss and German Gastro-

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TABLE 11 Liver biopsies in recurrent intrahepatic cholestasis of pregnancy (Category I)

Year	Author	Author's case number	Number of pregnancy	Time of biopsy (days)*	Duration of jaundice at biopsy (weeks)
1956	Ljunggren	?	1	early pp	?
				late pp	
1958	Gros	1	8	2 pp	6
1959	Svanborg & Ohlsson	?	?	ap	?
				pp	?
1959	Dolle & Martini	11	4	p	3
1960	Pieragnoli et al	1	2	3 pp	2
1961	Belvederi & Finotti	1	2	5 ap	4
		2	2	ap	4
1962	Cahill	1	2	?	3—4
		2	5	?	?
1962	Dietel	1	2	7 pp	3—4
1962	Hausheer & Lauer	1	4	1 pp	6
		1	5	10 pp	1
1963	Beraud et al	1	2	pp	4
1963	King & Kerrins	1	3	3 pp	3
1963	Moore	1	6	4 pp	4
		2	3	early pp	?
		2	4	at p	11
1964	Ikonen	L A	2	6 pp	?
		S V	5	ap	<1
		A S	2	14 pp	?
		A P	5	21 pp	<1
1966	Haemmerli & Wyss	B	2	21 pp	3
		C	2	3 pp	1
		E	3	2 pp	2
		F	6	at p	2

* ap = before delivery, pp = after delivery at p = on day of delivery

enterological Associations, September 30th 1960, in Zurich) and was first employed as title in a report describing own cases by Orellana and Osorio in 1963 The term is also used in Sherlock's textbook

Henceforward we will employ the terms 'intrahepatic cholestasis of pregnancy and recurrent intrahepatic cholestasis of pregnancy to designate a specific disease The term recurrent jaundice *during* pregnancy will be used as a descriptive one for the classification of diseases of different etiology

Findings	Needle biopsy	Surgical biopsy taken during	Abdominal operations without liver biopsy
Marked cholestasis	+		
Minimal cholestasis	+		
Mild cholestasis	+	(Peritoneoscopy)	
Cholestasis irregular and focal	+		
Normal	+		
Normal		*Caesarian section*	
Mild cholestasis	+		
Minimal cholestasis	+		
Centroacinar bile pigment in liver cells	+		
Cholestasis	+		
Cholestasis	+		
Cholestasis	+		
Minimal cholestasis	+		
Normal	+		
Focal cholestasis	+		Exploratory laparotomy T-drain in common duct
Cholestasis	+		
Normal	+		Interval cholecystectomy
Minimal cholestasis	+		
Minimal cholestasis		Caesarian section	
Cholestasis	+		
Normal		Abdominal hysterotomy	
Normal	+		Caesarian section
Normal		Abdominal hysterectomy	
Centroacinar bile pigment in liver cells		Exploratory laparatomy	Surgical sterilisation
Minimal cholestasis	+		
Mild cholestasis	+		
Minimal cholestasis		Abdominal hysterotomy	

Material for review of world literature

Whenever an attempt is made to delineate a newly emerging disease entity by reviewing all published case reports in the literature it appears wise to include in such a review only cases in which the diagnosis is beyond doubt In intrahepatic cholestasis of pregnancy no parameter singly or in combination with others is specific and none will provide an unquestionably correct diagnosis However two features will allow to restrict any errors in diagnosis to an acceptable minimum

TABLE 11 Liver biopsies in recurrent intrahepatic cholestasis of pregnancy (Category I)

Year	Author	Author's case number	Number of pregnancy	Time of biopsy (days)*	Duration of jaundice at biopsy (weeks)
1956	Ljunggren	?	1	early pp	?
				late pp	
1958	Gros	1	8	2 pp	6
1959	Svanborg & Ohlsson	?	?	ap	?
				pp	?
1959	Dolle & Martini	11	4	p	5
1960	Pieragnoli et al	1	2	5 pp	2
1961	Belvederi & Finotti	1	2	5 ap	4
		2	2	ap	4
1962	Cahill	1	2	?	3—4
		2	5	?	?
1962	Dietel	1	2	7 pp	3—4
1962	Hausheer & Lauer	1	4	1 pp	6
		1	5	10 pp	1
1963	Beraud et al.	1	2	pp	4
1963	King & Kerrins	1	3	5 pp	3
1963	Moore	1	6	4 pp	4
		2	3	early pp	?
		2	4	at p	11
1964	Ikonen	L A.	2	6 pp	?
		S V	5	ap	<1
		A S	2	14 pp	?
		A P	5	21 pp	<1
1966	Haemmerli & Wyss	B	2	21 pp	3
		C	2	3 pp	1
		E	3	2 pp	2
		F	6	at p	2

* ap = before delivery pp = after delivery at p = on day of delivery

enterological Associations, September 30th 1960, in Zurich) and was first employed as title in a report describing own cases by Orellana and Osorio in 1963. The term is also used in Sherlock's textbook.

Henceforward we will employ the terms 'intrahepatic cholestasis of pregnancy' and 'recurrent intrahepatic cholestasis of pregnancy' to designate a specific disease. The term 'recurrent jaundice *during* pregnancy' will be used as a descriptive one for the classification of diseases of different etiology.

Findings	Needle biopsy	Surgical biopsy taken during	Abdominal operations without liver biopsy
Marked cholestasis	+		
Minimal cholestasis	+		
Mild cholestasis	+	(Peritoneoscopy)	
Cholestasis, irregular and focal	+		
Normal	+		
Normal		Caesarian section	
Mild cholestasis	+		
Minimal cholestasis	+		
Centroacinar bile pigment in liver cells	+		
Cholestasis	+		
Cholestasis	+		
Cholestasis	+		
Minimal cholestasis	+		
Normal	+		
Focal cholestasis	+		Exploratory laparotomy T-drain in common duct
Cholestasis	+		
Normal	+		Interval cholecystectomy
Minimal cholestasis	+		
Minimal cholestasis		Caesarian section	
Cholestasis	+		
Normal		Abdominal hysterotomy	
Normal	+		Caesarian section
Normal		Abdominal hysterectomy	
Centroacinar bile pigment in liver cells		Exploratory laparatomy	Surgical sterilisation
Minimal cholestasis	+		
Mild cholestasis	+		
Minimal cholestasis		Abdominal hysterotomy	

Material for review of world literature Whenever an attempt is made to delineate a newly emerging disease entity by reviewing all published case reports in the literature it appears wise to include in such a review only cases in which the diagnosis is beyond doubt In intrahepatic cholestasis of pregnancy no parameter singly or in combination with others is specific and none will provide an unquestionably correct diagnosis However, two features will allow to restrict any errors in diagnosis to an acceptable minimum

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Year	Author	Author's case number	Number of pregnancy	Time of biopsy (days)*	Duratio of jaund at biops (weeks)
1956	Ljunggren	?	1	early pp	?
				late pp	
1958	Gros	1	8	2 pp	6
1959	Svanborg & Ohlsson	?	?	ap	?
				pp	?
1959	Dolle & Martini	11	4	p	5
1960	Pieragnoli et al	1	2	5 pp	2
1961	Belvederi & Finotti	1	2	5 ap	4
		2	2	ap	4
1962	Cahill	1	2	?	3–4
		2	5	?	?
1962	Dietel	1	2	7 pp	3–4
1962	Hausheer & Lauer	1	4	1 pp	6
		1	5	10 pp	1
1963	Beraud et al	1	2	pp	4
1963	King & Kerrins	1	3	5 pp	3
1963	Moore	1	6	4 pp	4
		2	3	early pp	?
		2	4	at p	11
1964	Ikonen	L.A.	2	6 pp	?
		S.V	5	ap	<1
		A.S	2	14 pp	?
		A.P	5	21 pp	<1
1966	Haemmerli & Wyss	B	2	21 pp	3
		C	2	3 pp	1
		E	3	2 pp	2
		F	6	at p	2

* ap = before delivery pp = after delivery, at p = on day of delivery

enterological Associations, September 30th 1960, in Zurich) and was first employed as title in a report describing own cases by Orellana and Osorio in 1963. The term is also used in Sherlock's textbook.

Henceforward we will employ the terms 'intrahepatic cholestasis of pregnancy' and 'recurrent intrahepatic cholestasis of pregnancy' to designate a specific disease. The term 'recurrent jaundice *during* pregnancy' will be used as a descriptive one for the classification of diseases of different etiology.

TABLE 12 *Liver biopsies in non recurrent intrahepatic cholestasis of pregnancy* (Category VII A)

Year	Author	Total cases	Histological diagnosis	
			Cholestasis	Normal
1947	Nixon et al. (case 14)	1	1	—
1953	Puyo (case 4)	1	1	—
1956	Ljunggren	6	5	1
1959	Svanborg & Ohlsson	4	3	1
1961	Katz et al.	4	3	1
1961	Van Woert & Kirsner	1	—	1
1963	Brown et al	3	3	—
1963	Myhre	1	—	1
1963	Orellana & Osorio	8	7	1
1964	Ikonen	2	1	1
1964	Gros	2	2	—
1966	Haemmerli & Wyss (unpublished)	2	2	—
	Combined	35	28	7

The only truly abnormal finding is confined to the centroacinar area Some *bile canaliculi contain bile plugs* The canaliculi are of normal caliber or occasionally slightly dilated The liver cells *surrounding the centroacinar bile canaliculi* contain bile pigment and may show an accumulation of fine basophilic *granules* *The portal tracts are not involved*

The histological picture is consistent with *the diagnosis of intrahepatic cholestasis* In only one case (Ljunggren) is cholestasis described as marked In 15 others bile *stasis is mild or even minimal* In 2 instances only biliary pigment in the centroacinar liver cells and no capillary bile plugs were found *In 5 cases* liver biopsy was described as normal Three authors have stressed the fact, that cholestasis, if present is irregular and focal (Svanborg and Ohlsson Beraud et al Haemmerli and Wyss) On different sections from the same biopsy *bile plugs* may be present in one slide and absent in the other (Haemmerli and Wyss) If cholestasis is not carefully looked for, it may be missed as often only a few bile thrombi are seen on one slide and bile pigment deposition in the liver cells is not impressive This may explain 3 normal readings the biopsies of which were taken before delivery in 1, *during* delivery in 1 and 4 days after delivery in a third case. The 2 other normal readings occurred in biopsies taken 14 *and* 21 days *after* delivery where cholestasis may well have disappeared

In 2 cases 2 biopsies were taken *during the same* pregnancy (Ljunggren Svanborg and Ohlsson) The cholestasis had regressed in both instances during the *second biopsy* taken after delivery In another case with a biopsy taken 3

1. recurrence of the same syndrome (defined on page 36) during successive pregnancies
2. a liver biopsy compatible with intrahepatic cholestasis

These two criteria are met in 14 reports covering a total of 23 patients. In only 18 of these sufficient information is available on clinical course and laboratory data. These 18 patients underwent a total of 70 pregnancies, 4 of which terminated in an early abortion, 9 were uncomplicated, 11 were associated with pruritus only and 47 showed the full syndrome with pruritus and jaundice. These 47 pregnancies will form the basis for our review.

A combined total of 132 cases of recurrent jaundice during pregnancy has been published in the literature. For the purpose of this review the cases have been divided into 6 categories according to their documentation (see Table 10). Occasionally it has become necessary to allot different cases contained in a single report into different categories.

Category I will be used to discuss liver biopsy findings, categories I A and II to discuss laboratory data. Categories I A and II A will serve for the description of the clinical course. The 61 cases in categories III, IV and V provide insufficient data for a critical evaluation. The 28 cases in category VI do definitely not belong to the disorder called intrahepatic cholestasis of pregnancy and will be discussed under the section on differential diagnosis.

The 267 patients with the non recurrent form of (possible) intrahepatic cholestasis of pregnancy (category VII) are added to complete the review. They will be discussed but briefly to point out some discrepancies with the confirmed cases or to point out some possible diagnostic pitfalls.

The reports covering category I and II cases originate from Sweden, Finland, England, Ireland, Germany, Switzerland, France, Italy, Poland, Canada, the United States of America and Chile. Additional countries contributing to category III—V cases are Holland, Austria, Roumania and Argentina and a category VII case is reported from Belgium.

Liver biopsies

In 25 pregnancies of 23 patients a total of 27 liver biopsies have been performed (category I, see Table 11). Of the 27 biopsies 20 were blind percutaneous needle biopsies, 1 consists of a needle biopsy taken during peritoneoscopy and 6 are surgical biopsies (Seydl's case with a normal biopsy 2 months after the disappearance of jaundice has been put into category II).

The findings are rather uniform. The liver architecture is intact. There is no liver cell damage. Neighbouring liver cells look alike. Minor deviations from the normal, consistent with those usually seen in uncomplicated pregnancy occur as an expression of an increased regenerative activity. They consist of Kupffer cell proliferation and mobilization, mild thickening of the framework, PAS positive granules in the Kupffer cells, some PAS containing macrophages in the portal spaces and of incipient ballooning of liver cells in the centroacinar areas.

normal biliary system at operation (*Moore case 1*)

The liver was considered to be of normal appearance in 10 instances and to be slightly enlarged in 1 patient (Ikonen, case A P) The spleen was found to be normal in all cases During peritoneoscopy Gros described the liver as being of normal size, shape and consistency, its surface as smooth, glistening, of grey brown colour with a greenish tint A close up view revealed a fine greenish stippling

One case in category I had gallstones (*Haemmerli and Wyss case F*) They were discovered after the 6th pregnancy No cholecystectomy was done This woman had only a single questionable episode of biliary colic 11 years previously and none during the next 16 years Three further patients of categories II—V had gallstones The patient of Katz et al (category II B) underwent a cholecystectomy for cholelithiasis after her 3rd pregnancy Her first pregnancy was asymptomatic her 2nd to 5th complicated by pruritus only and her 6th to 8th were associated with pruritus and jaundice Case O S of Ikonen (category IV) was cholecystectomized for gallstones after her 2nd pregnancy and was jaundiced again in her 3rd gestation Another patient with gallstones is Cahill s case 4 (category V no details)

In Moore s case 2 (category I A) the gallbladder was removed after her 6th pregnancy despite of a normal preoperative cholecystogram and despite of normal findings at operation Interestingly enough her next pregnancy was asymptomatic Her first 3 pregnancies were however asymptomatic too Non icteric pregnancies following icteric ones have been observed in 3 instances without cholecystectomy and without evidence of gallbladder disease (King and Kerrins, Ikonen case A S, Haemmerli and Wyss, case C)

These several observations make a causal relationship between gallstones and jaundice, or between cholecystectomy and absence of jaundice unlikely

Radiological gallbladder examinations were performed in 8 of the surgically explored cases and in an additional 6 patients without surgical procedures in category I Of the latter normal results were obtained in all (Cahill case 1, Pieragnoli et al King and Kerrins, Hausheer and Lauer Ikonen case L A, Haemmerli and Wyss case C) Of the patients with normal cholecystography after delivery three had a previous radiological examination during the height of jaundice, all showing non filling of the gallbladder (Beraud et al, Hausheer and Lauer, Haemmerli and Wyss, case C) In category II A cholecystograms were performed in 3 cases with normal results (Jodkowski and Chojecka, McAllister and Waddell, Simmons)

A very interesting observation was made in the case of Beraud et al In this woman jaundice set in in the second month of gestation and lasted until after delivery at term At 4 1/2 months of gestation an exploratory laparatomy was performed Despite normal macroscopic findings and a normal operative cholangiogram a T-drain was inserted into the common duct Bile flow from this tube was normal in volume, colour and viscosity and the intensity of jaun-

weeks after delivery biliary pigment was found in the centroacinar liver cells, but bile canaliculi were normal Apart from this rapid improvement after delivery, no correlation appears to exist between intensity of histological cholestasis and intensity of jaundice, duration of icterus before biopsy or time relation of biopsy to date of delivery

It is remarkable how little impressive histological cholestasis in this disease is when compared with the clear-cut clinical and biochemical "obstructive pattern", especially considering the often violent pruritus of the patients

A further 35 biopsies have been performed in the non recurrent form of intrahepatic cholestasis of pregnancy (category VII A, see Table 12) The histological findings were identical, with 28 biopsies showing cholestasis and 7 read as normal The 3 cases of so-called 'hepatitis" during pregnancy with normal liver biopsies reported by Ingerslev and Teilum in 1951 may represent 3 further examples

The 3 non-recurrent cases biopsied by Brown et al were examined by electron microscopy The microvilli of the bile capillaries were swollen and occupied most of the lumen Within the liver cells dilatation and vacuolization of the profiles of the endoplasmic reticulum was seen, with occasional complete desintegration of ergastoplasm No controls in uncomplicated pregnancy were done and no recurrent case of intrahepatic cholestasis of pregnancy has yet been examined by electron microscopy Furthermore two of the three cases were clinically atypical case 1 had splenomegaly and case 2 no pruritus and a prodromal phase of malaise, weakness and anorexia For these reasons, the findings of Brown et al cannot yet be accepted as representative for recurrent intrahepatic cholestasis of pregnancy

Biopsies have been performed in 6 other instances of recurrent jaundice during pregnancy (Nixon et al cases 16 and 19, Perreau and Rouchy, case II, Caroli et al case 3, Lebon et al, Justin—Besançon et al) The histological findings differed from those described above, but so did the clinical and biochemical findings These cases do not represent intrahepatic cholestasis of pregnancy and will be discussed under the section on differential diagnosis

Gross anatomical findings and radiological gallbladder examinations

No woman died of the disorder and no post mortem examinations have been performed

Ten of the 23 patients in category I underwent 11 surgical abdominal interventions, in 7 of which liver biopsies were taken (see Table 11) The interventions consisted of 3 surgical interruptions of gestation in the 3rd or 4th month because of jaundice (Ikonen cases S V and A P, Haemmerli and Wyss, case F) 2 exploratory laparatomies because of jaundice (Beraud et al, Haemmerli and Wyss case B) 1 peritoneoscopy because of jaundice (Gros) 3 Caesarian sections (Moore case 2, Dolle and Martini, Ikonen case V S) 1 surgical sterilisation on the day of spontaneous delivery (Haemmerli and Wyss case B) and 1 cholecystectomy in the interval between 2 pregnancies with

TABLE 14 Clinical course in recurrent intrahepatic cholestasis of pregnancy Cases without liver biopsy but with adequate laboratory data (category II A)

YEAR	AUTHOR	Case	Pregnancy	WEEKS OF GESTATION 4 8 12 16 20 24 28 32 36 40 44
1954	SVANBORG	1	1	
			2	
			3	
		2	1	
			2	
			3	
		3	1	
			2	
		4	1	
			2	
1961	JODKOWSKI + CHOJECKA	1	1	
			2	
1961	PERREAU + ROUCHY	IV	1	
			2	
			3	
		V	1	
			2	
			3	
			4	
		VI	1	
			2	
			3	
			4	
			5	
		IX	1	
			2	
			3	
			4	
			5	
			6	
			7	
			8	
1962	McALLISTER WADDEL	1	1	
			2	
			3	
1962	SEYDL	1	1	
			2	
1963	MOORE	3	1	
			2	
1963	SIMMONS		1	
			2	
1966	HAEMMERLI WYSS	A	1	
			2	
		D	1	
			2	
			3	
			4	

TABLE 13 Clinical course in recurrent intrahepatic cholestasis of pregnancy Cases verified by liver biopsy (category I A)

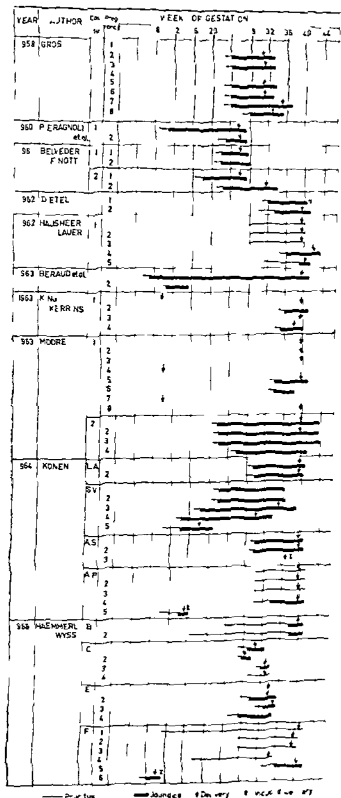


TABLE 14 Clinical course in recurrent intrahepatic cholestasis of pregnancy. Cases without liver biopsy but with adequate laboratory data (category II A)

YEAR	AUTHOR	Case no.	Pregnancy	WEEKS OF GESTATION: 4 · 8 · 12 · 16 · 20 · 24 · 28 · 32 · 36 · 40 · 44
1954	SVANBORG	1	1	
			2	
			3	
		2	1	
			2	
			3	
		3	1	
			2	
		4	1	
			2	
1961	JODKOWSKI + CHOJECKA	1	1	
			2	
1961	PERREAU + ROUCHY	IV	1	
			2	
			3	
		V	1	
			2	
			3	
			4	
		VII		
			2	
			3	
			4	
			5	
		IX	1	
			2	
			3	
			4	
			5	
			6	
			7	
			8	
1962	McALLISTER WADDEL	1	1	
			2	
			3	
1962	SEYDL	1	1	
			2	
1963	MOORE	3		
			2	
1963	SIMMONS			
			4	
1966	HÄMMERLI WYSS	A	1	
			4	
		D	1	
			2	
			3	
			4	

Table 15 Clinical course in pruritic recurrent jaundice during pregnancy. Cases without adequate laboratory data. Possibly examples of recurrent intrahepatic cholestasis of pregnancy (category III)

YEAR	AUTHOR	Case nr	Preg nr	WEEKS OF GESTATION 4	8	12	16	20	24	28	32	36	40	44
1881	AHLFELD		1											
1898			2											
			3											
			4											
1896	BECKING		1											
			2											
			3											
			4											
			5											
1903	BAUER VON		1											
1904	DEN VELDEN		2											
			3											
			4											
1906	MAYER		1											
			2											
1924	BOREEL		1											
			2											
			3											
			4											
			5											
1929	MAGNANI		1											
			2											
			3											
1932	SCHWALM		1											
			2											
			3											
			4											
			5											
			6											
			7											
			8											
			9											
			10											
			11											
			12											
1940	ERHAGE	4												
			2											
			3											
			4											
			5											
			6											
			7											
			8											
1953	PERREAU	I												
1961	ROUCHY													
1962	GABRIEL													
	BERNARD		2											
			3											
			5											
1964	IKONEN	P												
			2											
			3											
		AM												
			2											
			3											

dice did not change during the long drainage period

Symptoms and signs

The disease has been observed in all age groups during the childbearing period The clinical course in categories I A, II A and III is graphically summarized in Tables 13, 14 and 15 respectively

Pruritus is the first symptom to occur and dominates the clinical picture throughout the whole course in this disorder Pruritus is usually violent and involves the trunk or the extremities or both. Most authors note that the palmar and plantar surfaces of hands and feet may be involved When pruritus lasts for more than two weeks excoriations (scratch marks) usually appear on the skin In a rare case they may become secondarily infected and result in an impetigonous rash (Haemmerli and Wyss first pregnancy of case F) Pruritus usually leads to insomnia which renders the women tired and irritable Pruritus precedes jaundice usually by 1—2 weeks but may begin up to 22 weeks earlier (Haemmerli and Wyss, second pregnancy of case B)

In some instances pruritus is the only clinical symptom of liver dysfunction and jaundice does not occur at all In these cases pruritus may be of long duration (up to 30 weeks in first pregnancy of case F by Haemmerli and Wyss) Pruritus gravidarum without jaundice appears to be a forme fruste of intrahepatic cholestasis of pregnancy

Intrahepatic cholestasis of pregnancy has been called jaundice of late pregnancy and it has been stated that jaundice always begins during the last 4 months of gestation (Svanberg and Ohlsson) This applies to the majority of the cases but there are many exceptions Mean and median onset of jaundice among 47 icteric pregnancies in category I A is in the 26th week of gestation or just before the beginning of the last trimester The observed range of onset varies between the 7th and the 39th week of gestation with 2 pregnancies in the 7—8th week, 2 in the 11th, 3 in the 14th, 2 in the 18th 14 in the 22nd to 24th 1 in the 26th, 9 in the 28th to 30th, 7 in the 32nd to 34th, 4 in the 35th to 36th and 3 pregnancies in the 38th to 39th week of gestation

In 7 cases of category I A jaundice was noted between the 7th and 14th week of gestation In addition jaundice began in the third month of gestation during the second to fifth pregnancy in Cahill s case 2 (category I B) Of 37 icteric pregnancies in category II A onset of jaundice was in 5 between the 10th and 16th week, in 5 between the 20th and 24th week, in 8 between the 26th and 28th week, in 8 between the 30th and 32nd week, in 7 between the 33rd and 36th week and in 4 between the 37th and 40th week. Category III includes possibly other forms of jaundice besides intrahepatic cholestasis of pregnancy Still it may be of interest to note that icterus began in all 12 pregnancies of Schwalm s case in the first month of gestation Onset of jaundice in the 3rd month occurred in 5 pregnancies of Boreel s and in 3 pregnancies of Magnani s case

Of course statistical figures dating onset of jaundice are in a way fallacious.

TABLE 15 Clinical course in pruritic recurrent jaundice during pregnancy Cases without adequate laboratory data Possibly examples of recurrent intrahepatic cholestasis of pregnancy (category III)

WEEKS OF GESTATION: 4 8 12 16 20 24 28 32 36 40 44

YEAR	AUTHOR	Case	Pregnancy
1881 1898	AHLFELD		1
			2
			3
			4
1896	BECKING		1
			2
			3
			4
			5
1903 1904	BAUER VON DEN VELDEN		1
			2
			3
			4
1906	MAYER		1
			2
1924	BOREEL		1
			2
			3
			4
			5
1929	MAGNANI		1
			2
			3
1932	SCHWALM		1
			2
			3
			4
			5
			6
			7
			8
			9
			10
			11
			12
19 0	VERHAGE	4	1
			2
			3
			4
			5
			6
			7
			8
1953 961	FERRE ... ROUCHY		
195?	GABRIEL BERNARDIN		3
1964	IKONEN	I P	1
			2
			3
		A M	1
			2
			3

may be difficult to elicit On the other hand, liver and spleen size were normal in all 10 cases with surgical abdominal explorations Easy bruising or echymosis would be expected in cases with prolonged prothrombin time (usually occurring in cases with jaundice of long duration) but has never actually been observed in these patients

In no case of categories I and II did onset of jaundice precede onset of pruritus and in no case did jaundice disappear before delivery After delivery whether spontaneous or induced, jaundice decreases rapidly and disappears usually within 1—2 weeks In 46 instances in category I A jaundice disappeared within 1 week in 18 within 2 weeks in 21 within 3 weeks in 1 and within 4 weeks in 6 instances The 6 instances with prolonged post-delivery jaundice are confined to 2 patients Moore s case 2 and Ikonen s case S V Moore s case 2 forms an exception in another respect the intensity of jaundice increased during the first postpartum week The same phenomenon occurred once in a case of category II A (Jodkowski and Chojecka second pregnancy) Jaundice lasted for 6 weeks after delivery in 3 pregnancies of the non verified case of Brauer and Von den Velden (category III)

Pruritus disappears before jaundice subsides The only dissenting opinion is that of Svanborg and Ohlsson All other authors agree that pruritus may disappear completely on the first postpartum day in some cases and decreases markedly in intensity immediately after delivery in all other instances to disappear completely before jaundice vanishes.

There is no evidence that intrahepatic cholestasis of pregnancy leaves any residual liver damage, but long term and careful follow up examinations have been few in number Slightly elevated alkaline phospatase levels have been observed 4 months and 1 year after an icteric pregnancy in 2 cases (Haemmerli and Wyss cases B and F), but repeat examinations later on were normal In one case bromsulfalein retention was 24 % in 45 minutes 2 months after delivery (Beraud et al) and in another 7 % 1 year after delivery (Ikonen, case S V) Long term follow up examinations of 1 to 5 years with normal results were obtained in 10 cases (Thorling cases 39 and 56 Svanborg and Ohlsson 3 cases, Cahill case 1, Haemmerli and Wyss cases A B D and F) Pavel s case 1 (category IV) developed probable hepatitis 3 months after the last of 6 gestations with jaundice, but recovered rapidly and was well at the age of 65 years

Laboratory data

Laboratory data of patients in category I A and category II A are summarized in Tables 16 and 17 respectively The values given are the most abnormal ones recorded in the individual pregnancy for each single test. The several test results given for a single individual were not necessarily obtained on the same day For example peak bilirubin levels were usually observed before delivery but peak alkaline phosphatase levels a few days after delivery Furthermore, few patients have been serially examined and the maximal abnormal tests summarized in the tables may well be submaximal

The patient herself will notice her jaundice often well after its true onset On the other hand serum bilirubin elevation was noted very early before onset of clinical jaundice in at least 3 instances with onset of jaundice in the first trimester These patients were carefully followed by their physicians because of the history of their previous pregnancies with jaundice (Ikonen cases S V and A P, Haemmerli and Wyss, case F)

Duration of jaundice from its observed onset until spontaneous delivery varied in 42 instances of category I A between 1 and 33 weeks, with a mean of 8 1 and a median of 6 weeks Duration of 1 to 2 weeks occurred in 7 instances, of 3 to 4 weeks in 7, of 5 to 6 weeks in 8, of 7 to 8 weeks in 6, of 10 to 11 weeks in 4, of 12 to 13 weeks in 4, of 15 to 18 weeks in 5 and of 33 weeks in 1 instance

In category II A duration of jaundice from its observed onset until spontaneous delivery varied in 33 instances between 1 and 24 weeks, with a mean of 7 7 and a median of 5 weeks Duration of 1 to 2 weeks occurred in 9 instances, of 3 to 4 weeks in 6, of 5 weeks in 3, of 8 to 9 weeks in 6, of 12 to 16 weeks in 5 and of 17 to 24 weeks in 4 instances

During jaundice or just preceding its onset the urine is described as dark by all patients The stool colour is light in some instances, definitely normal in others, and not noticed by the patient or physician in most cases

The general health of the patients is not impaired during the jaundiced stage They feel well — apart from the irritation and lack of sleep resulting from itching — and continue without difficulties their daily work, including heavy manual labor (farmer's wife) This is in marked contrast to the reduced general condition in viral hepatitis In intrahepatic cholestasis of pregnancy there are no prodromal or general symptoms such as fever, nausea, vomiting, liver pains, arthralgias, anorexia — again in contrast to the clinical picture in viral hepatitis

The 3 cases reported in the two Italian papers are the only ones to have associated symptoms apart from pruritus and jaundice The first symptom during the first pregnancy in all 3 cases was severe pain in the right upper quadrant of the abdomen, radiating to the right shoulder in two cases In the patient of Pieragnoli et al and in case 1 of Belvederi and Finotti this was associated with fever up to 38° centigrade These symptoms are probably not related to intrahepatic cholestasis of pregnancy and did not recur in the two cases during the second icteric pregnancy Case 2 of Belvederi and Finotti had some pains in the right upper abdominal quadrant during two pregnancies A cholecystogram was normal in the case of Pieragnoli et al The 2 cases of Belvederi and Finotti had the same gastrointestinal symptoms already before their first pregnancy

No woman in categories I and II presented evidence of toxemia of pregnancy Toxemia and pyelitis were noted in Ikonen s case O S (category IV)

Physical examination is unrevealing except for the presence of jaundice and scratch marks The liver is rarely and the spleen never palpable As examination of these patients is usually performed near term palpatory findings

Transaminases Wróblewski units		Prothrombin time %	Thymol turbidity MacLagan units	Zinc sulfate flocc Kunkel units	Cephalin cholest flocculation	Takata reaction	Serum iron γ/100 ml	Bromsulfalein retention %	Galactose tolerance test
SCOT	SGPT								
			2				68		
								↑	
25		53			–			↑	
25		53	N		–			↑	
		42	N						
↑↑	↑↑	100				+	109		
34		100	5 7		(+)				
50			N		0				
		100	7					22	
		70	2		0			25	N
920		89	1		0				
			3						
			2						
			N						
200			1						
107	230	33					110		
75	100								
95									
76	160							18	
30	22	60			0	N	50–205		N
69	50		2.2	3 2	0		50–100	10	N
79	53	70			0	N	45–130		
52	53	100	4 9	7 9	0	N	115–180		
238	175	50	4 5	4 7	0		130		
90	90	85			0	N	20–190		
106	145				0	N	40–140		
142	2 0		3 5		0		90–155		
183	226	30			0	N	130–250	13	N
1 0	197	82	4 4	6 4	0	N	355		

* Conversion from King Armstrong units.

case VI and 8 4 mg per 100 ml (McAllister and Waddell). The direct reacting serum bilirubin fraction determined in 10 instances of category IA is responsible for most of the total serum bilirubin elevation. Bilirubinuria was found in 17 of 20 instances. Bilirubinuria may be short lasting or intermittent, and can therefore easily be missed (Thorling Haemmerli and Wyss). Urobilinogen is

Table 16 Recurrent intrahepatic cholestasis of pregnancy
Cases with detailed laboratory data and liver biopsy (Category I A)

Year	Author	Number of case and pregnancy	Urine			Serum Bilirubin mg/100 ml		Alkaline phosphatase Bodansky units	Cholesterol mg/100 ml
			Bilirubin	Urobilin	Urobilinogen	Total	Direct		
1958	Gros	1—8	+		↑	4 6	2 9	N	221
1960	Pieragnoli et al	1—2	+	+		6 8	5 0		400
1961	Belvederi & Finotti	1—2	+	+		6 8	5 0		400
		2—2	+	+		5 3	4 0		386
1961	Dietel	1—2	+	—		3 5		13*	280
1962	Hausheer & Lauer	1—4				4 5	1 9	22*	314
		1—5				3 2	1 3	15*	
1963	Beraud et al	1—1	+			5 5	4 1		213
		1—2	+			5 6	4 2	28	240
1963	King & Kerrins	1—2				3 5	2 2		
		1—4				3 7	3 0	6 8	
1963	Moore	1—6				2 1		28	
		2—4				3 5		26	
1964	Ikonen	L A —2	+			2 8			
		S V —5				1 2		7	255
		A S —2				1 5			515
		A S —3				0 5		4	400
		A.P —4	+			3 5		6 8	
		A P —5	—			1 2		4 2	
1966	Haemmerli & Wyss	B —1	(+)	—	N	3 5		11 6	287
		B —2	—	—	N	3 6		21 2	590
		C —1	+	+	N	5 9		17 5	290
		C —2	+	—	↑	3 6		14 6	450
		C —3	+	+	↑	3 6		14 4	420
		E —2	+	—	N	4 2		11 4	
		E —3	+	—	N	4 5		13 4	360
		E —4	(+)	—	N	4 5		11 8	300
		F —5	(+)	(+)	N	5 0		12 6	333
		F —6	↑	—	↑	3 1		12 7	200

N = normal ↑ = increased + = positive — = negative * Conversion from Bessey Lowry units

because examinations have not been performed during the peak abnormality

Serum bilirubin determinations have been obtained in 29 pregnancies of the 18 patients in category I A Jaundice is always of mild degree The maximal recorded value was 6 8 mg per 100 ml This value is surpassed three times in category II A with 7 8 (Haemmerli and Wyss, case A), 8 0 (Perreau and Rouchy

Transaminases Wróblewski units		Prothrombin time %	Thymol turbidity MacLagan units	Zinc sulfate flocc. Kunkel units	Cephalin cholesterol flocculation	Takata reaction	Serum iron γ/100 ml	Bromsulfalein retention %	Galactose tolerance test
SGOT	SGPT								
			2				68	↑	
25		53			—			↑	
25		53	N					↑	
		42	N						
↑↑	↑↑	100				+	109		
34		100	5.7		(+)				
50			N		0			22	
		100	7					25	N
		70	2		0				
970		89	1		0				
			3						
			2						
			N						
200			1						
107	230	33					110		
5	100								
95									
76	160							18	
50	22	60			0	N	50–205		N
69	50		2.2	3.2	0		50–100	10	N
9	53	70			0	N	45–130		
52	53	100	4.9	7.9	0	N	115–180		
238	175	50	4.5	4.7	0		130		
90	90	85			0	N	20–190		
106	145				0	N	40–140		
142	220		3.5		0		90–155		
183	226	30			0	N	130–250	13	N
150	197	82	4.4	6.4	0	N	355		

* Conversion from King Armstrong units.

case VI and 8.4 mg per 100 ml (McAllister and Waddell). The direct reacting serum bilirubin fraction determined in 10 instances of category IA is responsible for most of the total serum bilirubin elevation. Bilirubinuria was found in 17 of 20 instances. Bilirubinuria may be short lasting or intermittent, and can therefore easily be missed (Thorling; Haemmerli and Wyss). Urobilinogen is

TABLE 17 Recurrent intrahepatic cholestasis of pregnancy
Cases with detailed laboratory data, but no liver biopsy (*Category II*)

Year	Author	Number of case and pregnancy	Urine: Bilirubin	Urine: Urobilin	Urine: Urobilinogen	Serum Bilirubin mg/100ml: Total	Serum Bilirubin mg/100ml: Direct	Alkaline phosphatase Bodansky units	Cholesterol mg/100 ml
1954	Svanborg	1–2				3.8		10^{+}	
		1–3				2.6		7^{+}	
		2–1				3.5		28^{+}	
		2–3				5.1		16^{+}	
		3–2				2.6		10^{+}	
		4–2				1.6		8^{+}	
1955	Thorling	39–3				2.1		6^{+}	
		51–2				3.0		14^{+}	
		56–1				2.3		14^{+}	
1961	Jodkowski & Chojecka				N	6.5		11.8	235
1961	Katz et al					3.6	1.4	15	334
1961	Perreau & Rouchy	IV—3	+			2.6			342
		V—4	+			4.0			460
		VI—2	+			8.0			170
		VIII—5	+			2.5		9*	388
		IX—8	+			6.8			240
1962	McAllister & Waddell		+			8.4	3.0	6.6*	
1962	Seydl					3.1		15	
1963	Moore	3–1				2.6	1.9	8*	
		3–2				3.9		16*	
1963	Simmons	1–1				2.5		15*	
		1–2	+			3.0		19*	
1966	Haemmerli & Wyss	A–2				7.8		6	231
		D–1	(+)	+	↑	3.6		20.7	213
		D–2	—	+	↑	2.4			384
		D–3	—	—	N	1.3		22.0	370
		D–4	—	—	↑	0.8		19.4	350

N = normal ↑ = increased + = positive — = negative * Conversion from King Armstrong units

present in normal or increased amounts, but is never completely absent from the urine. Urobilin was found in 6 of 14 instances.

Consistent with the clinical and histological feature of cholestasis there is as a rule an elevation of alkaline phosphatase and cholesterol. One or the other or even both these tests may be normal, however, in an occasional case.

Alkaline phosphatase, determined in 22 pregnancies of 14 patients in category I A was normal in 2 pregnancies between 4 and 7 Bodansky units in 4

Transaminases Wróblewski units		Prothrombin time %	Thymol turbidity MacLagan units	Zinc sulfate flocc Kunkel units	Cephalin cholest flocculation	Takata reaction	Serum iron γ/100 ml	Bromsulfalein retention %	Galactose tolerance test
SGOT	SGPT								
			5			↘			
			2			↘			
			1						
			1			↘			
			3			N			
			1			↘			
		115	1			+			
		89	N	N	0				
		96	3						
		^				+			
5?	94	100	N			N		16	
		100							
		77							
80	48		8		2+			16	
	↑	80							
					0				
			1						
			2						
			3						
			N	2					
			2	2					
						↘			N
		37			+	N	45–160		
91	77				+	N	55–160	23	↘
66	,8	100	3	5 1	(+)	↘	100–160	23	
28	27				0	N	100–200		

+ Conversion from Buch Buch units.

pregnancies and between 11 and 28 Bodansky units in 16 gestations The findings in category II A correspond in general to the ones in category I A As most figures given for category II A had to be converted into Bodansky units from other units no data will be tabulated Serum cholesterol, determined in 13 pregnancies of category I A was below 250 mg per 100 ml in 3 and between 255 and 590 mg per 100 ml in 10 gestations In 12 pregnancies of category II A serum cholesterol varied between 170 and 460 mg per 100 ml.

The elevation of serum cholesterol is seldom more pronounced than that seen in uncomplicated pregnancies (Ikonen), whereas serum alkaline phosphatase elevation is — when present — usually much more marked than the usual elevation observed towards term in uncomplicated gestations (Thorling) There is no correlation in the single case between elevation of serum bilirubin, alkaline phosphatase or cholesterol

Serum electrophoresis, performed in 15 pregnancies of 5 patients reported by Haemmerli and Wyss, fits also into the pattern of biochemical cholestasis There is a decrease in serum albumin, a slight increase of alpha-1 and a moderate increase of alpha-2 globulins, a usually pronounced increase in beta globulins and normal or slightly decreased gamma globulins In every case the beta globulin fraction is higher than the gamma-globulin fraction The electrophoretic changes represent an exaggeration of those seen in uncomplicated pregnancy Total proteins are somewhat decreased as in normal gestation

The serum turbidity and flocculation tests are normal as a rule, with few, but only borderline exceptions (Perreau and Rouchy, case VIII)

Prothrombin time is normal or only slightly prolonged in the majority of cases It may be prolonged to critical levels whenever jaundice is of long duration This is the only test that shows such a correlation Of 17 pregnancies in category I A prothrombin time was below 60 % in 7 instances, 3 of which being around 30 % Prolongation of prothrombin time is entirely due to a deficiency in the Vitamin K dependent coagulation factors II, VII and X (Haemmerli and Wyss) and is readily corrected by the application of Vitamin K

Serum glutamic oxalacetic transaminase may be normal or increased In 21 pregnancies of 12 patients in category I A it was normal in 4, between 50 and 100 units in 9, between 100 and 240 units in 7 patients and 920 units in the case reported by King and Kerns Serum glutamic pyruvic transaminase, determined in 14 pregnancies of 7 patients, was normal in 1, between 50 and 100 units in 6 and between 100 and 230 units in 7 gestations It was not determined in the case with the exceptionally high SGOT Besides the high SGOT found in the case of King and Kerns, 2 other recurrent cases of Ikonen (not specified which ones) had similar high levels SGOT 716 and SGPT 875 in one case and SGOT 450 and SGPT 565 in the other SGPT was higher than SGOT in 9 of 16 cases with both determinations (category I A and Ikonen's cases) Five cases in category II with determination of the transaminases show mild to moderate elevations

The 3 cases with very high transaminases are astonishing As they fit in all other regards into the general picture of intrahepatic cholestasis of pregnancy, and as one of these cases has been biopsied, they are tentatively accepted in this review For practical diagnostic purposes, however, it appears wise for the present to regard a level of 250 units as the upper limit usually seen in this disease

Serum 1 phospho-fructaldolase was elevated to 8 4 units and serum sorbit

dehydrogenase up to 7 6 units in case E of Haemmerli and Wyss In serial determinations during two pregnancies of this patient both enzymes paralleled the behaviour of the serum transaminases closely

Bromsulfalein retention is always increased during jaundice The recorded values in 8 cases of category I A and 4 cases in category II varied between 10 % and 25 % after 45 minutes A special modification using a single intravenous injection of 800 mg bromsulfalein was employed in the three Italian cases of category I A This test revealed a decreased hepatic uptake of bromsulfalein and an increased cholestatic index in all

In contrast to the increased bromsulfalein retention urinary galactose excretion after a 40 g oral loading dose is always normal (4 tests in category I A and 2 in II A in 4 of 6 tests simultaneous bromsulfalein test)

There is no evidence of hemolysis in this disorder No erythrocyte survival tudies have been performed and a mild hemolysis as a contributary phenomenon is therefore not excluded Hemoglobin is usually normal for pregnancy The lowest recorded hemoglobin levels are 63 % (Hausheer and Lauer) 66 % (Haemmerli and Wyss second pregnancy of case B) and 67 % (Jodkowski and Chojecka) Reticulocyte counts are not increased Urobilinogenuria cannot be used as a criterium for hemolysis in the presence of disturbed liver function Serum iron levels behave erratically (Ikonen Haemmerli and Wyss) with both abnormally low and abnormally high levels (up to 355 microgm per 100 ml) in the same pregnancy Nearly all increased levels were found before delivery, decreased levels both before and after There is no correlation between serum iron and hemoglobin There is also no correlation between serum iron and serum transaminases such as occurs in viral hepatitis (Haemmerli and Wyss)

Total leucocyte and differential counts are normal or show a leucocytosis and/or shift to the left as in uncomplicated pregnancies Non protein nitrogen is never elevated.

Laboratory data before and after delivery

Long and serial pre delivery laboratory examinations have been performed only in the 6 cases reported by Haemmerli and Wyss These periods were of 14, 15 22 24 62 and 125 days duration It is probable but not conclusively proved that an elevation of the serum transaminases first indicates the onset of the full syndrome However bilirubin elevation lags but a few days behind Serial observations in cases with a long preicteric pruritic phase are necessary to settle this question. Serum alkaline phosphatase and serum cholesterol are usually elevated before the serum bilirubin or the transaminases The significance of this elevation is difficult to evaluate as both these parameters are increased in the last trimester of most normal pregnancies

In cases with jaundice of short duration serum bilirubin usually increases towards delivery In cases with jaundice of long duration serum bilirubin rapidly reaches a plateau It may remain steady or fluctuate and even decrease slightly

The elevation of serum cholesterol is seldom more pronounced than that seen in uncomplicated pregnancies (Ikonen), whereas serum alkaline phosphatase elevation is — when present — usually much more marked than the usual elevation observed towards term in uncomplicated gestations (Thorling) There is no correlation in the single case between elevation of serum bilirubin, alkaline phosphatase or cholesterol

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Table 18 Recurrent intrahepatic cholestasis of pregnancy
Cases with liver biopsies but no details on clinical course (category I B)

YEAR	AUTHOR	CASE NR	PREGNANCIES
1956	LJUNGGREN		■ ■
1959	SVANBORG + OHLSSON		■ ■
1959	DOLLE + MARTINI		■ ■ ■ ■
1962	CAHILL	1	■ ■
		2	□ ■ ■ ■ ■

Table 19 Recurrent intrahepatic cholestasis of pregnancy
Cases with laboratory data but no details on clinical course (category II B)

YEAR	AUTHOR	CASE NR	PREGNANCIES
1955	THORLING	39	□ □ ■ ■
		51	■ ■
		56	■ ■
1961	KATZ et al		□ ■ ■ ■ ■ ■ ■ ■
1961	PERREAU + ROUCHY	VI	■ ■ ■

Legend for Tables 18 and 19 see Table 20

43 pregnancies with jaundice and spontaneous delivery in the 18 cases of category I A there were 23 premature deliveries and 20 deliveries at or near term However this kind of statistics is treacherous in a way because 22 premature deliveries occurred in 7 women and 19 deliveries at term in 10 women In these cases every delivery of the single individual was either at term or premature (see Table 13 In just one case is a premature delivery combined with a delivery at term (Beraud et al

These general conclusions are also evident in category II A Premature delivery correlates neither with time of onset of jaundice in relation to stage of pregnancy nor with the intensity or the duration of jaundice Furthermore premature delivery appears to be independent of intrahepatic cholestasis of pregnancy itself Case E of Haemmerli and Wyss delivered prematurely in 4 pregnancies, the first of which was uncomplicated Similarly case C of Haemmerli and Wyss delivered prematurely

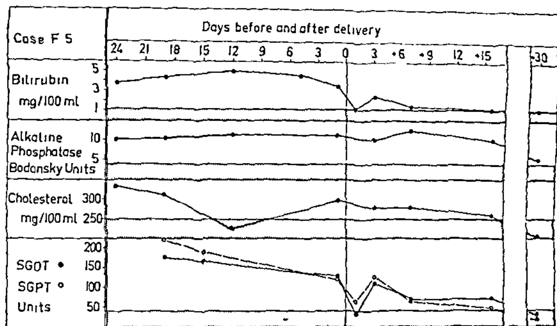


Fig 1 Example of laboratory data in recurrent intrahepatic cholestasis of pregnancy (case F fifth pregnancy, Haemmerli and Wyss 1966)

toward delivery (Haemmerli and Wyss, case A)

After delivery serum bilirubin and serum transaminases begin to decrease immediately and are, together with urinary bile pigments, the first tests to become normal again Serum alkaline phosphatase may continue to increase for 4 to 10 days after delivery (Haemmerly and Wyss) and is the last test to become normal Mild elevations may persist for up to 2 months after delivery

A curious phenomenon was observed by Haemmerli and Wyss in 2 cases Immediately after delivery there was a transient "dip" or return to normal of serum bilirubin and serum transaminases, only to reach pre-delivery levels again within 24 hours A gradual improvement occurred thereafter (see Fig 1) It it not clear whether this phenomenon is a rule or an exception Because of its short lasting transient nature it will easily be missed if serial laboratory determinations in 8 to 10 hour intervals are not performed during the first day after delivery

Obstetrical course, incidence of premature deliveries and child survival

No obstetrical complications occurred during delivery in category I A There is a single mild complication in category II A patient D reported by Haemmerli and Wyss had a blood loss of 750 ml during her first delivery Her prothrombin time was 37 % of normal The child's prothrombin time was 0 % and it died despite immediate Vitamin K therapy from massive intracranial hemorrhage The bleeding in the mother stopped spontaneously and did not necessitate blood transfusions

The incidence of premature deliveries is high in the combined series Among

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Cases with liver biopsies but no details on clinical course (category I B)

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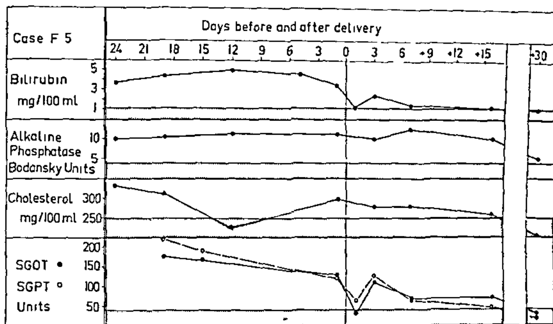


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		2	□ ■ ■ ■ ■

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Legend for Tables 18 and 19 see Table 20

43 pregnancies with jaundice and spontaneous delivery in the 18 cases of category I A there were 23 *premature* deliveries and 20 deliveries at or near term However this kind of statistics is treacherous in a way because 22 premature deliveries occurred in 7 women and 19 deliveries at term in 10 women In these cases every delivery of the single individual was either at *term or premature* (see Table 13) In just one case is a premature delivery combined with a *delivery at term* (Beraud et al)

These general conclusions are also evident in category II A Premature delivery *correlates* neither with time of onset of jaundice in relation to stage of pregnancy nor with the intensity or the duration of jaundice Furthermore *premature delivery* appears to be independent of intrahepatic cholestasis of pregnancy itself Case E of Haemmerli and Wyss delivered prematurely in 4 pregnancies *the first of* which was uncomplicated Similarly case C of Haemmerli and Wyss delivered prematurely

Table 20 Recurrent jaundice during pregnancy
Cases reported with few details (category IV)

YEAR	AUTHOR	CASE NR	PREGNANCIES
1902	MULLERHEIM		□ ◨ ◨
1910	NASON		□ □ A ◨ ◨ ◨ ◨ ◨ □ □ □
1910	ROLLESTON		■ ■ ■ ■
1924	BOREEL	mother of case	◨ ◨ ◨ ◨ ◨ ◨ ◨
1935	PEL		◨ ◨ □ ◨ ◨
1953	PUYO	2	■ ■ ■
1954	DOWIE		◨ ◨
1955	MEYER	I	◨ ◨
		2	◨ ◨
1957	PAVEL et al	I	■ ■ ■ ■ ■ ■
		2	■ ■ ■ A ◫ ■ ◫
1958	WILKEN	2	◨ ◨
1959	SVANBORG + OHLSSON		■ ■ ■
			■ ■
			■ ■
		sister	■ ■
1961	PERREAU + ROUCHY	VII	◨ A ◨
		sister of case VI	■ ■ ■ ■ ■ ■
1961	ORELLANA et al		■ ■
			■ ■ ■
1961	SHEEHAN		■ ■ ■
1963	ORELLANA + OSORIO		■ ■ ■ ■
			□ □ ◫ ◫ ■ ■ ■
1964	IKONEN	O S	■ ■
		L V	■ ■
		M S	□ ■ ■

□ uncomplicated pregnancy
◫ pregnancy with pruritus
■ pregnancy with pruritus and jaundice
◨ pregnancy with jaundice pruritus not mentioned not negated
A abortion

in 4 pregnancies the last of which was complicated by pruritus without jaundice

Thus premature delivery appears to be a feature of the single individual The overall high incidence remains still to be explained Perhaps whatever mechanism causes intrahepatic cholestasis of pregnancy may independently also cause premature delivery, with no direct causal relationship between jaundice and premature delivery

Of 23 children born prematurely 10 died shortly after birth Of the 20 children born after the 36th week 6 died during delivery mainly from intrauterine asphyxia The already mentioned death of an infant with a prothrombin time of 0 % from intracranial bleeding and a similar incident in a non recurrent case of Thorling s are probably unrelated to the jaundiced mother's coagulation deficiency because extremely low prothrombin times are encountered not infrequently in newborns from healthy mothers

No baby was jaundiced in categories I and II Several jaundiced babies were observed in 3 non verified cases of category IV (Nason Rolleston Pavel et al case 1) Erythroblastosis foetalis is not excluded in these cases, 2 of which were reported in 1910 There were 2 congenital malformations in category III both occurring in the same mother (case I P Ikonen) the first child died with ileal atresia 6 weeks after birth and the second was stillborn with a ventricular septal defect Jaundice began during the 5th month in the first and during the 8th month in the second gestation so that a causal relationship to the malformations is very unlikely

TABLE 21 Recurrent jaundice during pregnancy Cases without any details reported (Category V)

Year	Author	No of cases
1872	Hoffman	1
1905	Kehrer	4
1923	Eppinger	1
1927	Holmer	1
1937	Wewalka	5
1961	Meeroff	1
1962	Cahill (cases 3 and 4)	2
1962	Friedberg	4
1963	Orellana and Osorio	2
1964	Ikonen	4
	Total	23

Clinical course of successive pregnancies in the individual patient

The pregnancies of categories I A, II A and III are summarized in Tables 13 14 and 15 respectively, the pregnancies of categories I B II B and IV in Tables 18 19 and 20 Category V cases without details are mentioned in Table 21

A glance at Tables 13 and 14 will rapidly show that the clinical course in this disease is by no means uniform About the only existing rule is that jaundice occurs some time before delivery and disappears rapidly thereafter, whether delivery is spontaneous or induced

It has been stated by Svanborg and Ohlsson that the disease runs a similar course in the individual patient in repeated pregnancies This generalization is true only to a certain extent and is

TABLE 20 Recurrent jaundice during pregnancy
Cases reported with few details (category IV)

YEAR	AUTHOR	CASE NR	PREGNANCIES
1902	MULLERHEIM		□ ◫ ◫
1910	NASON		□ □ A ◫ ◫ ◫ ◫ ◫ □ □ □
1910	ROLLESTON		■ ■ ■ ■
1924	BOREEL	mother of case	◫ ◫ ◫ ◫ ◫ ◫ ◫
1935	PEL		◫ ◫ □ ◫ ◫
1953	PUYO	2	■ ■ ■
1954	DOWIE		◫ ◫
1955	MEYER	1	◫ ◫
		2	◫ ◫
1957	PAVEL et al	1	■ ■ ■ ■ ■ ■
		2	■ ■ ■ A ▤ ■ ▤
1958	WILKEN	2	◫ ◫
1959	SVANBORG + OHLSSON		■ ■ ■
			■ ■
			■ ■
		sister	■ ■
1961	PERREAU + ROUCHY	VII	◫ A ◫
		sister of case VI	■ ■ ■ ■ ■ ■
1961	ORELLANA et al		■ ■
			■ ■ ■
1961	SHEEHAN		■ ■ ■
1963	ORELLANA + OSORIO		■ ■ ■ ■
			□ □ ▤ ▤ ■ ■ ■
1964	IKONEN	O S	■ ■
		L V	■ ■
		M S	□ ■ ■

□ uncomplicated pregnancy
▤ pregnancy with pruritus
■ pregnancy with pruritus and jaundice
◫ pregnancy with jaundice pruritus not mentioned not negated
A abortion

serum bilirubin levels of 3 6, 2 4 1 3 and 0 8 mg per 100 ml in 4 successive pregnancies

In category V there are 2 remarkable cases In the patient of Dowie the interval between the first and second pregnancy with jaundice was 12 years The case of Nason had jaundice only in the 4th to 8th of her 11 pregnancies In the 7th and 8th pregnancy jaundice disappeared about 10 days before delivery, so that the possibility of diagnosis other than intrahepatic cholestasis of pregnancy exists

No definite statement as to the frequency of recurrence in successive pregnancies can be made because non recurrent cases have been excluded from this survey Thorling reported 10 multiparas with gestation after an icteric pregnancy Three had again pruritus and jaundice two pruritus only and 5 were asymptomatic during the next pregnancy

Treatment

Intrahepatic cholestasis of pregnancy is a benign disorder with full recovery after delivery One hesitates even to call it liver disease From the standpoint of therapeutic consequences it might as well be regarded as just a disordered function of little importance Neither bed rest nor a dietary regimen are necessary and the patient may continue her normal daily life Whenever jaundice is of longer duration (more than 2 weeks) the prothrombin time should be checked regularly If this is not practicable Vitamin K can be given prophylactically

Pruritus the most annoying symptom does not respond to antihistaminics (Gros, Haemmerli and Wyss) Itching is easily abolished with cholestyramine, an ion exchange bile acid sequestrant, in the dose of 10 gm per day When the drug is stopped pruritus recurs within 1 to 2 days (Haemmerli and Wyss) Cholestyramine was also successfully employed in the non recurrent case 3 of Brown et al and in a case of pruritus gravidarum with slightly elevated alkaline phosphatase, cholesterol and transaminases but no jaundice (Haemmerli and Wyss unpublished observation)

Intrahepatic cholestasis of pregnancy is definitely not an indication for an induced termination of pregnancy An induced termination of pregnancy may lead to the loss of the child It will certainly cure the mother's jaundice but this will be cured anyhow after spontaneous delivery As pruritus can now be easily controlled with cholestyramine there is no symptom which could demand a shortening of the jaundiced period Today induced terminations of pregnancy are usually performed by physicians who are not familiar with the disorder and mistake it for a serious liver disease

Antecedent or underlying hepato biliary or gastrointestinal disease

The past history of patients with intrahepatic cholestasis of pregnancy is usually non revealing except for the usual childhood diseases Case B of Haemmerli and Wyss (category I A) had probable viral hepatitis at the age of 8 years Nonspecific upper gastrointestinal disturbances before the first pregnancies were noted for 1 and 9 years respectively in the 2 cases of Belvederi and Finotti

even contradicted by the 4 cases reported by Svanborg in detail 5 years earlier (category II A) An apparent similarity exists in the course of 8 of 18 cases in category I A However, among these 8 cases only the one of Belvederi and Finotti is reported with exact details of all pregnancies In the other 7 reports (Gros, Dietel, Hausheer and Lauer, 4 cases of Ikonen) the descriptions of all except the last pregnancy of the cases are lumped together (for example "Jaundice usually appeared in the 24th week and lasted until 2 weeks after delivery"), and therefore we assume that the "similarity of the course" is more fictitious than real If a history on previous pregnancies is obtained during the last pregnancy only, not to much weight should be put upon a woman's recollection Wherever detailed charts for each pregnancy were available for retrospective review (as in the series of Haemmerli and Wyss) similarity is less striking

Jaundice does not necessarily occur during every pregnancy of the single individual Jaundice in every pregnancy was present in only 9 of 18 cases in category I A Seven of these 9 cases underwent 2 pregnancies only and may therefore not be representative for the full spectrum of possibilities in this disease

Jaundice when present is not necessarily of the same intensity during successive pregnancies Jaundice of equal intensity was noticed in the same 9 of 18 cases mentioned above 7 patients with 2 pregnancies 1 with 4 (Moore case 2) and one with 5 pregnancies (Ikonen, case S V)

The other 9 patients in category I A show a varying symtomatology during successive gestations It may be significant, that all these cases underwent 3 or more gestations (mean 5 2, maximum 8)

In the patient reported by King and Kerrins only the second and the fourth pregnancy were associated with pruritus and jaundice, whereas the first and third were uncomplicated In case 1 of Moore only 2 of 6 fullterm pregnancies were symptomatic, the first three and the last one being uncomplicated

In 5 cases the syndrome appears to progress in intensity during successive pregnancies In 3 patients the first 3 or 4 pregnancies were complicated by pruritus only, the following two showing the full syndrome with jaundice (Hausheer and Lauer, Ikonen case A P Haemmerli and Wyss case F) In the other 2 cases the first pregnancy was asymptomatic, with the full syndrome in the following gestations (Gros Haemmerli and Wyss case E) In addition Cahill's case 2 (category I B) conforms to this pattern, but no details are given

In 2 cases a definite improvement is noted in successive pregnancies with the last one being completely asymptomatic (case A S by Ikonen case C by Haemmerli and Wyss)

Among the 15 cases in category II A (Table 12) jaundice was about equal in successive pregnancies in 12 patients 8 of which with 2 3 with 3 and 1 with 4 pregnancies A progression in intensity is shown in the cases VIII and IX by Perreau and Rouchy with the first several pregnancies asymptomatic or pruritic only An instructive example of decreasing intensity is provided by case D of Haemmerli and Wyss with peak

serum bilirubin levels of 3 6, 2 4, 1 3 and 0 8 mg per 100 ml in 4 successive pregnancies

In category V there are 2 remarkable cases In the patient of Dowie the interval between the *first* and *second* pregnancy with jaundice was 12 years The case of Nason had jaundice only *in the* 4th to 8th of her 11 pregnancies In the 7th and 8th pregnancy jaundice disappeared about 10 days before delivery, so that the possibility of diagnosis other than intrahepatic cholestasis of pregnancy exists

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These symptoms recurred during their pregnancies (category I A) Ulcerative colitis of about 1 year's duration was diagnosed in the middle of the 12 year interval between the 2 pregnancies with jaundice of Dowie's case (category IV)

The 4 patients with cholelithiasis have already been mentioned in the paragraph on radiological gallbladder examinations

Familial occurrence of intrahepatic cholestasis of pregnancy

Some patients with recurrent intrahepatic cholestasis of pregnancy have close relatives with a history of recurrent jaundice during pregnancy None of these relatives has been examined in detail

Jaundice during pregnancy occurred in 2 sisters of one of Cahill's cases (category I B or V, not stated which case) Case VI of Perreau and Rouchy (category II A) had a sister with jaundice during 6 successive pregnancies, each leading to premature delivery Pruritus and jaundice were observed during one of several pregnancies in the mother of the case reported by both Brauer and by von den Velden (category III) The mother of Boreel's case (category III) was jaundiced during 7 pregnancies and the patient was the only surviving child all others dying from prematurity Two pregnancies with jaundice occurred in the older sister of a patient reported by Svanborg and Ohlsson (category IV)

The mother of case S V reported by Ikonen (category I A) had pruritus without jaundice during the 4th 5th 10th and 11th of her 13 pregnancies Pruritus with jaundice occurred once in a maternal cousin of the same case

The two sisters with recurrent jaundice during pregnancy reported by both Benedict and Lovrich probably represent another disorder as both had hepatomegaly and one a marked splenomegaly in addition An older sister and the mother of these two cases had uncomplicated gestations

In Mayer's case (category III) the mother died from liver carcinoma and the father and one sister suffered from "chronic liver disease"

An inherited predisposition to intrahepatic cholestasis of pregnancy can neither be accepted nor be excluded from these observations

Non recurrent intrahepatic cholestasis of pregnancy

A total of 267 cases with a possible diagnosis of non recurrent intrahepatic cholestasis of pregnancy have been published in the literature The laboratory data are summarized in Table 22 In 55 cases liver biopsies were performed (see Table 12) Associated cholelithiasis thought to be unrelated to jaundice was found in 17 of 267 patients

Many of these cases are incompletely documented and many may belong to quite different diagnostic categories In 15 patients jaundice was associated with severe pyelonephritis (Van Woert and Kirsner Laurijssens and Demeulenaere Comerford, case 4 Thorling case 42 Ikonen 11 cases) and in 3 cases jaundice was associated with pyelitis and hemolysis (Thorling cases 40, 41 and 43 Toxemia of pregnancy was present in

case 10 of Comerford and in 27 of Ikonen's cases. Two cases of Thorling had marked *hypertension* (number 64 and 67). Ten patients of Ikonen had anemia. Nine cases had no pruritus (Thorling 3 cases, Svanborg and Ohlsson 5 cases, Brown et al case 2). Six cases listed are definitely not examples of intrahepatic cholestasis of pregnancy. In 4 of Thorling's cases jaundice disappeared well *before delivery*. Myhre's *case* had no pruritus, severe hypertension, marked hepatomegaly, an elevated serum amylase and a normal liver biopsy. Brown et al's case 1 had splenomegaly.

On the other hand 8 cases published as viral hepatitis in the original reports have been included in Table 22 on the *basis of their clinical and biochemical* data (Nixon et al case 14, Barry and O'Dwyer case 1, Comerford cases 1, 3, 5, 6, 7 and 9).

In Thorling's excellent monography on 72 patients with jaundice during pregnancy he writes: "Purely on the basis of the clinical signs and symptoms in the individual cases the diagnosis of viral hepatitis appears to be possible in practically all the patients." He selected from this group 35 cases on two criteria: jaundice appeared in late pregnancy and the *thymol turbidity test was negative*. While the majority of this group may truly represent intrahepatic cholestasis of pregnancy, many will not. Wewalka pointed out that the thymol turbidity test may *not be reliable during pregnancy*. In his series thymol turbidity was positive in 82 % of patients with hepatitis outside of pregnancy, but in only 53.5 % of 58 cases with serum hepatitis (treatment for syphilis) during pregnancy.

The data in Table 22 are given for what they are worth. Just a few details will be pointed out.

It is remarkable that Orellana and *Osorio collected their 59 cases within* 2 years in a single hospital. Serum bilirubin levels are below 6.8 mg per 100 ml *in all cases with one exception* (case 2 of Brown et al without pruritus, 8.7 mg per 100 ml). Alkaline phosphatase, cholesterol, prothrombin time, turbidity and flocculation reactions correspond to those in verified cases of recurrent intrahepatic cholestasis of pregnancy. The one high bromsulfalein retention of 50 % is from *Brown et al's case 1 with splenomegaly*. The biopsied case 2 of Gros had a 39 % retention, whereas all other *bromsulfalein retention tests performed* are between 14 and 28 % as in the verified cases. It will be remembered that in the verified category I A and II A cases the serum transaminases were below 250 units with three exceptions (King and Kerrins, Ikonen 2 cases). High values were also found in the non-recurrent *case 3 of Brown et al* (SGOT 670, SGPT 220).

It must be concluded from this brief survey that the non-recurrent form of intrahepatic cholestasis of pregnancy is extremely difficult to diagnose with absolute certainty. The disease can easily be simulated by viral hepatitis with a benign course occurring late *in pregnancy* or by drug-induced intrahepatic cholestasis, except that both these diseases have typical prodromal symptoms. For a diagnosis of non-recurrent intra*hepatic cholestasis* of pregnancy an absence of "hepatitis-like" prodromi and the rigid clinical, biochemical and histo-

TABLE 22 Non recurrent intrahepatic cholestasis of pregnancy (267 cases)
Diagnosis mostly not verified (Category VII)

Year	Author	Number of cases	Number of biopsies	Number of cholecystograms: Total	Stones	Urine bilirubin	Serum bilirubin mg/100 ml: Total	Direct
1947	Nixon et al (case 14)	1	1				3 5	1 4
1953	Puyo (cases 4 & 6)	2	1			+	1 6–5 0	
1954	Svanborg	3		2	2		2 1–5 1	
1955	Barry & O Dwyer	1					2 0	
1955	Thorling	35		17	2		<5 8	
1956	*Ljunggren*	51	6	34	5		<6 5	
1959	Svanborg & Ohlsson	18	4	18	4		2 0–6 0	
1961	Katz et al.	7	4				<5 4	~3 0
1961	Orellana et al	12					2 2–6 8	0 5–2 4
1961	Van Woert & Kirsner	1	1			+	2 2	1 5
1962	Comerford	6					1 6–5 0	
1962	Laurijssens & Demeulenaere	1					4 6	
1963	Brown et al	3	3				2 2–8 7	1 3–2 8
1963	Moore	6						
1963	Muller & Felsch	20						
1963	Myhre	1	1	1			3 5	2 1
1963	Orellana & Osorio	59	8	21		+	1 3–6 8	0 3–3 1
1964	Ikonen	35	2	35	4	+	1 0–5 0	^
1964	Gros	2	2	1		+	2 0–3 5	
1966	Haemmerli & Wyss (unpublished)	3	2			+	2 4 5 8	

N = normal ^ increased + = positive ° *Conversion from Buch Buch units* ⁺ *Conversion from*

logical criteria emerging from the review of the verified recurrent cases (category I A) must be demanded

Pruritus is the prominent and probably compulsory symptom but the presence of itching alone does not make the diagnosis of intrahepatic cholestasis of pregnancy Pruritus appears to occur *more* frequently in all liver diseases during pregnancy than in liver disease outside of pregnancy Thorling found pruritus in 17 of 23 cases of viral hepatitis during pregnancy (74 %) and believes that the pregnant state in some way favours the manifestation of this symptom in the presence of hepatitis Martini et al observed itching in 12 of 57 cases of hepatitis during pregnancy Pruritus occurs also in hyperemesis gravidarum with jaundice and may be present in this disease without jaundice (Thorling) In two cases of acute fatty

Alkaline phosphatase Bodansky units	Cholesterol mg/100 ml	Transaminases Wróblewski units SGOT	SGPT	Prothrombin time %	Thymol turbidity	Different flocculation tests	Bromsulfalein retention %
	630				N↘	↘	
8–30°					↘	↘	
14				↑	N↘		
6–28°				3 ↑	N	N↘	
~20°				4 50–70	↘		
				8 <50			
<26°				7 ↑	↘	↘	
<16+	<310	<76	<148	>55	↘	↘	14–24
12–36	215–331			83–100	↘	↘	
15	250			100	N↘	N↘	
6 21+					↘		
15		40	25		↘		
13 14+	<279	55–670	62–220	↘	↘		28–50
		30–260	17–297				
11+		112	80	N	N	↘	
9–36	29>300			13↑	N	N↘	
5–20		2>300	2>300	20–100	4↑		
5 8		26–68	57–75	100	↘	↘	39
11–18	270–330	45–246	39–340	↘	↘	↘	

° King Armstrong units   + Conversion from Bessey Lowrey units.

metamorphosis of pregnancy marked pruritus was noted (Ober and Lecompte case 1 CPS case) Itching was also a feature in the two cases of jaundice with hemoglobinuria during pregnancy (Schaeffer Meinhold)

Pruritus gravidarum

Itching has been defined as an unpleasant cutaneous sensation which provokes the desire to scratch (Thorling) or as one of the seemingly mild symptoms which seems humorous to observers but which may be desperately serious to the patient' (Kasdon) Itching is the most frequent cutaneous disturbance during pregnancy and occurs in a generalized or localized form the latter as abdominal vulvar or anal pruritus Both forms usually occur during the second half of gestation

Abdominal pruritus has been noted by Kasdon in 7 % of 42 women in the first trimester, in 20.9 % of 110 women during the second trimester and in 18.3 % of 213 women during the third trimester. The etiology is unknown.

The term pruritus gravidarum should be reserved for the generalized form. No statistics exist as to its frequency, but the syndrome is well known to all obstetricians. Circumstantial evidence favors the concept that pruritus gravidarum is a *forme fruste* of intrahepatic cholestasis of pregnancy. First of all, many cases of recurrent intrahepatic cholestasis of pregnancy will present with recurrent pruritus without jaundice during their first few gestations. Well documented examples have been published by Katz et al., Perreau and Rouchy case IX, Hausheer and Lauer, Orellana and Osorio, Ikonen case A. P. and Haemmerli and Wyss case F. Secondly, in all cases of intrahepatic cholestasis of pregnancy jaundice is preceded and accompanied by pruritus. Thirdly, pruritus gravidarum has its onset in the second or third trimester and disappears with or shortly after delivery, i.e. its clinical course in relation to stage of gestation is the same as the course of intrahepatic cholestasis of pregnancy. Lastly, cases of pruritus gravidarum show some disturbed liver functions which lie between those observed as physiological derangements during normal pregnancy and those seen in intrahepatic cholestasis of pregnancy.

Arfwedson 1953 and 1956 compared 100 pregnancies with pruritus gravidarum to 100 without. Serum bilirubin in those with pruritus was between 1 and 2 mg per 100 ml in 42 % and above 2 mg per 100 ml in 23 %, compared to only 6 % of determinations greater than 1 mg per 100 ml in normal pregnancy. Pathological bile components were found in 51 % of those with pruritus gravidarum and in 5 % of normal pregnancies. In 1956 Arfwedson and von Studnitz examined 42 women with pruritus gravidarum and 46 without during the last trimester. In those with pruritus there was a significant increase in total serum lipids, serum cholesterol, phospholipids and beta lipoproteins, whereas alpha lipoproteins were decreased. On serum electrophoresis there was a decrease in albumin and an increase in alpha 2 and beta 2 globulins in those with pruritus. Borglin found a SGOT elevation to 93 units in 1 out of 7 cases. Serum ornithyl carbamyl transferase was increased in 24 of 30 patients with pruritus (80 %) compared to only 15 % pathological results in normal pregnancies (Reichard et al.).

Few detailed case studies have been performed. In Thorling's 3 cases (number A, B and C) the serum bilirubin, thymol turbidity and cephalin flocculation were normal and the alkaline phosphatase was increased up to 35 Buch and Buch units. Laboratory data in the 2 cases reported by Topp and Charles were: urine bilirubin positive, serum bilirubin 1.5 mg per 100 ml, alkaline phosphatase 35 King—Armstrong units and bromsulfalein retention 30 % in 35 minutes in case 1; urine bilirubin positive, serum bilirubin 1.0 mg per 100 ml, alkaline phosphatase 62 King—Armstrong units, cholesterol 295 mg per 100 ml, bromsulfalein retention 35 % at 35

minutes, transaminases, prothrombin time and flocculation tests normal in case 2 In an unpublished observation of Haemmerli and Wyss (wife of the second author) the following laboratory data were obtained no abnormal urine bile components total bilirubin 0 9 mg per 100 ml direct reacting bilirubin 0 3 mg per 100 ml, alkaline phosphatase 11 2 Bodansky units, serum cholesterol 330 mg per 100 ml, SGOT 69 units SGPT 63 units, 1 phosphofructaldolase 4.5 units (normal up to 2 8 units) normal results for prothrombin time serum iron and thymol turbidity

Therapeutically testosteron effectively controls itching but increases serum bilirubin levels (Arfwedson and von Studnitz In the case of Haemmerli and Wyss cholestyramine in moderate doses (6 gm per day) brought complete relieve but itching recurred upon each attempted cessation of the drug Cholestyramine was also successfully used in a case reported by Brown et al

Pruritus gravidarum once it occurs has a marked tendency to recur in successive pregnancies (Thorling Topp and Charles case 2) and may lead to intrahepatic cholestasis of pregnancy in a later gestation (Bjerregaard Perreau and Rouchy case III)

3) Differential diagnosis of recurrent intrahepatic jaundice during pregnancy

The 28 cases in category VI are all examples of recurrent jaundice during pregnancy in which intrahepatic cholestasis of pregnancy can be excluded with near certainty on the basis of the published data It is likely that some cases in categories III, IV and V would also have to be classified under a different heading if more data were provided for a critical evaluation of the orignal reports

In 15 of the 28 cases the author's original diagnosis has been accepted in this review In 8 cases a tentative reclassification was made (Perreau and Rouchy case II, Puyo cases Le B and B S, Nixon et al case 16, Tylecote, Lantuejoul and Chambraud, Ezes and Bourdon, Lebon et al) and 5 cases could not be classified at all (Benedict Lovrich Vignes, Boquien et al, Justin—Besançon et al) Sometimes our reclassification will appear arbitrary and occasionally different opinions as to the etiology of a particular case may be possible even where a liver biopsy has been performed For these reasons the case reports are recorded in some detail in this chapter

Recurrent jaundice during pregnancy due to recurrent viral hepatitis or due to exacerbation of chronic anicteric hepatitis

Until recent years most French authors were convinced that recurrent jaundice during pregnancy is in all *instances* caused by viral hepatitis (Caroli et al) In no case such an etiology is proved In three cases in which liver biopsies were performed it may be possible

The patient of Albano and Albano contracted viral hepatitis during her first pregnancy After typical prodromal symptoms she developed jaundice in the 7th month with a large tender liver serum bilirubin up to 27 6 mg per 100

ml (direct reacting bilirubin 20 7 mg per 100 ml), both serum transaminases up to 1,100 units, positive zinc sulfate and thymol turbidity tests, normal alkaline phosphatase and bilirubinuria She delivered several weeks later and was discharged with mild subicterus 2 weeks after delivery In the interval she had some dyspeptic symptoms but no jaundice Seven months later she was pregnant again During the first month fever, dyspepsia, liver pains and subicterus set in In the 5th month the serum bilirubin was 1 9 and the direct reacting bilirubin 1 0 mg per 100 ml The urine was positive for bilirubin and urobilinogen, and there was an increase in gamma-globulins on electrophoresis Alkaline phosphatase, the transaminases and all flocculation and turbidity reactions were normal By the 7th month bilirubin had risen to 10 3 mg per 100 ml, SGOT to 300 units, SGPT to 400 units and the flocculation reactions were now positive Liver biopsy showed an intact lobular architecture and massive round cell infiltration of the periportal spaces On bed rest and medical treatment including steroids she improved, delivered at term and has since been anicteric

It is possible that jaundice during the second pregnancy in this case is due to either a true recurrence of the initial viral hepatitis, dormant after the first pregnancy or that it represents a clinical manifestation of an anicteric chronic hepatitis under the stress of pregnancy

Case II reported by Perreau and Rouchy had pruritus followed by jaundice in the 3rd month of her first two pregnancies, both times with premature deliveries in the 6th month and subsidence of jaundice afterwards During the second pregnancy bilirubinuria was present, serum bilirubin was 19 8 mg per 100 ml (direct reacting bilirubin 14 9 mg per 100 ml), akaline phosphatase was 8 6 Bodansky units, prothrombin time was normal and the flocculation reactions were negative A liver biopsy 9 days after delivery showed dislocated hepatic cell plates, enlarged liver cells with clarification of the cytoplasm, hyperplasia of the Kupffer cells and a mild increase in connective tissue This was interpreted as "diffuse hepatitis" Jaundice disappeared 3 weeks after delivery and she was asymptomatic during the next 4 years She then became pregnant for the third time and developed pruritus followed by jaundice in the 5th month Serum bilirubin was 15 8 mg per 100 ml, bilirubinuria was present, and the cephalin flocculation was 1 + She delivered at 7 1/2 months and jaundice disappeared one week later Six months after delivery she had a large and firm spleen a serum bilirubin of 1 5 mg per 100 ml and no excretion of contrast material during radiological gallbladder examination At present she has the full picture of liver cirrhosis verified by liver biopsy (personal communication from Dr Perreau)

This case clearly developed toward liver cirrhosis while presenting with jaundice during 3 successive pregnancies Again, chronic hepatitis is most likely the underlying primary disease

Another possible example of chronic underlying liver disease after hepatitis with exacerbation during pregnancy is a case published by Puvo (Mme Le B

observation of Cachera also published by Caroli et al and by Lacomme) This woman had viral hepatitis of mild degree at age 22 At age 23 she became icteric during the 4th month of her first pregnancy and had dark urine and light stools She delivered at 8 1/2 months and jaundice disappeared one month later During her second pregnancy she became jaundiced in the 4th month delivered in the 6th and was cured one month afterwards Six months later she had an enlarged firm liver and spleen a serum bilirubin of 3 2 mg per 100 ml and a positive cephalin flocculation Liver biopsy showed hyperplasia of the architecture of the liver cell plates During her third pregnancy she was again jaundiced before her delivery at term

Recurrent jaundice of pregnancy due to incipient primary biliary cirrhosis

A case published by Tylecote in 1914 may well represent an example of primary biliary cirrhosis This woman underwent 8 pregnancies during the age of 18 to 32 years In each pregnancy there was an insidious onset of jaundice with bilirubinuria during the 3rd month of gestation She usually delivered prematurely during the 7th or 8th month of gestation and jaundice did not clear until 3 months after delivery Since the last pregnancy she remained permanently jaundiced her liver was markedly enlarged and massive xanthomatosis appeared With the rapid succession of her multiple pregnancies and the long duration of jaundice after delivery it is possible that this woman was more or less permanently jaundiced already before the onset of her xanthomatosis

No definite diagnosis can be made in this case because eight pregnancies would be very unusual in primary biliary cirrhosis Ahrens et al reported 17 females with this disease, 10 of which had an uncomplicated pregnancy prior to the clinical manifestation of their cirrhosis Three were pregnant during their illness In two of these bilirubin levels were not followed at that time while in one there was a sharp drop of serum bilirubin and serum lipids during pregnancy, to rise again after a spontaneous abortion in the 4th month

Recurrent jaundice during pregnancy due to posthepatitic hyperbilirubinemia

Posthepatic hyperbilirubinemia thoroughly discussed by Kalk on the basis of 165 cases is a syndrome indistinguishable from the familial Gilbert s syndrome except for the presence of viral hepatitis in the past history It is characterized by a mild elevation of the indirect reacting serum bilirubin fraction with a normal direct reacting bilirubin and normal results in all other tests used to evaluate liver function

Dietel has published a case with posthepatitic hyperbilirubinemia during 2 pregnancies (case E Fr) Two years after a first normal pregnancy this woman had typical viral hepatitis During the next two pregnancies 2 and 4 years after her hepatitis respectively she became mildly jaundiced during the last trimester with an elevation of the indirect serum bilirubin to between 2 7 and 3 5 mg per 100 ml Hemolysis was excluded and all other liver tests were

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A second case of Dietel's represents posthepatitic hyperbilirubinemia during one pregnancy only. This woman contracted viral hepatitis one year after her first normal pregnancy. Two years after the hepatitis she became pregnant for the second time and developed mild jaundice during the last trimester with elevation of the indirect serum bilirubin fraction to between 2.5 and 3.5 mg per 100 ml. Liver biopsy in this case was normal and jaundice cleared rapidly after delivery. Martini et al. mention three similar cases without giving any details.

Recurrent jaundice during pregnancy due to gallstones in the common bile duct

It has already been mentioned that jaundice due to common duct stones is extremely rare during pregnancy. It is not astonishing, therefore, that no documented cases of recurrent jaundice during prenancy due to this condition exist. Two cases may possibly fall into this category. Rissmann described in 1910 a patient with several episodes of biliary colic before her first pregnancy. One week before term during her first pregnancy she had a similar attack followed by jaundice which disappeared after delivery. Jaundice with colicky pains in the right upper abdominal quadrant occurred again two weeks before delivery during her second pregnancy. Another example may be case 1 (Mme B. S.) reported by Puyo and again by Caroli et al. This woman was jaundiced during the 6th month of her first pregnancy. Jaundice disappeared after delivery in the 7th month. The patient was plagued with intermittent colicky pains in the gallbladder area ever since. During the next pregnancy she was jaundiced from the 7th month until after her delivery at term. Cholelithiasis was found on radiological examination and a cholecystectomy was performed some months later. During the third pregnancy she had again an episode of jaundice lasting 4 weeks during the 4th month of gestation, but this time it cleared well before delivery.

Recurrent jaundice during pregnancy due to familial non hemolytic jaundice

Four cases of this syndrome have been reported in the literature. In all hemolysis has been excluded with a reasonable degree of certitude, but in none an exact diagnosis could be established (such as Gilbert's syndrome, the Rotor syndrome or the Dubin—Johnson syndrome).

Paschkis reports a case with "constitutional hyperbilirubinemia" and jaundice during 12 successive gestations. A case of "Cholemie familiale" published by Chabrol, a pupil of Gilbert, had subicterus and an enlarged spleen when not pregnant and "extremely intensive jaundice" with epistaxis and melena during 3 successive gestations, each time subsiding after spontaneous abortion. Two patients with chronic mild jaundice and a history of the same disorder in their fathers and in one instance in a twin sister are reported by Ikonen. Case E. K. had jaundice with a serum bilirubin of 5.8 mg per 100 ml in her otherwise uncomplicated first pregnancy. The next

two pregnancies terminated in spontaneous abortions The chronic subicterus deepened again during the 4th gestation, the patient developed some epigastric pain and the liver margin became palpable and tender There was no bilirubinuria and the thymol turbidity was negative A bromsulfalein clearance was interpreted as pointing to the Dubin—Johnson syndrome Liver biopsy was refused and a cholecystogram normal Case H F with chronic subicterus had first an abortion in the 3rd month During the second pregnancy the serum bilirubin was 2 6 *mg per 100 ml* and no bilirubin was present in the urine She aborted in the 5th month *In the third pregnancy serum bilirubin* was 2 0 mg per 100 ml alkaline phosphatase 4 3 Bessey—Lowry units and SGOT 59 *units* Urine bilirubin was negative Premature delivery took place *in the 7th* month One year later the patient became febrile and jaundiced Radiological examination revealed gall stones

Recurrent jaundice during pregnancy due to hemolysis

Two examples of hemolysis with jaundice due to unknown cause are reported in the literature The first seven pregnancies in the case of Bromberg et al were uncomplicated During the 8th gestation headache dizziness anorexia and jaundice were noted in the 7th month Hemoglobin was 6 1 *mg per 100 ml* reticulocyte counts markedly elevated and total serum bilirubin 2 2 mg per 100 ml with a negative direct Van den Bergh *reaction* Urine bilirubin was absent and urobilinogen increased The woman delivered at term and recovered rapidly thereafter During the next four years *no acute hemolytic crisis* occurred, but her hemoglobin remained around 7 5 to 9 gm per 100 ml, reticulocyte counts re*mained elevated and the spleen* became enlarged She became pregnant for the 9th time and suffered from an acute *hemolytic crisis in the 25th week* with a drop in hemoglobin to 4.5 gm per 100 ml and a rise in serum bilirubin to 4 6 *mg per 100 ml* After induced abortion she recovered rapidly Radiological gallbladder examination was normal

The case of Zachariae had a *normal* first pregnancy followed by one with an abortion She was anemic during her *third gestation (hemoglobin 51 %)* Towards the end of her 4th pregnancy the hemoglobin dropped to 37 % and jaundice with a *serum* bilirubin *of* 5 0 *mg* per 100 ml was present until delivery 3 weeks later Splenomegaly was noted During the next 2 years she had *once* a hemolytic episode without jaundice while suffering from thrombophlebitis During the fifth pregnancy hemolysis with jaundice occurred in the third month and was cured by an induced abortion

A third case of recurrent jaundice during pregnancy is due to congenital spherocytosis (Rimbach and Beickert) The patient s father the father's brother and mother had chronic mild jaundice while the *patient herself* was never jaun*diced outside her pregnancies During* 4 consecutive gestations *she developed* hemolytic crisis with jaundice the hemo*globin dropping* each time to about 30 % Each time hemolysis disappeared within 2 weeks after delivery *The spleen*

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was enlarged and morphological blood examination was consistent with spherocytosis

A fourth case, reported by Schneider and Frahm, has a mixed etiology This woman had anemia during her 3rd and 4th pregnancy, and hemolytic anemia with mild jaundice during her 5th and 6th pregnancy Investigation revealed a large spleen and a megaloblastic bone marrow After the cure of her pernicious anemia with Vitamin B 12 the red cell morphology was typical for congenital spherocytosis, which subsequently was discovered to exist also in 3 of her siblings

Recurrent jaundice with hemoglobinuria during pregnancy

In 1902 both Schieffer and Brauer reported the case of an Italian woman with marked pruritus and mild jaundice in the second half of 6 successive pregnancies except during the third which was terminated early by a spontaneous abortion General symptoms were mild and the jaundice cleared rapidly after delivery The dark urine contained bilirubin, urobilin and in addition hemoglobin A similar non-recurrent case was observed by Meinhold in 1903 in a primipara, with marked pruritus and hemoglobinuria, but without jaundice and without bile constituents in the urine Both patients inhabited areas were malaria was common both had no fever and both had negative tests for malaria

No further case of pregnancy with hemoglobinuria has been reported since It is therefore unlikely that such an entity exists in reality

Recurrent jaundice during pregnancy due to severe pyelonephritis

The only acceptable case of recurrent jaundice during pregnancy due to pyelonephritis in the literature is the one published by Fruhinsholz in 1929 During the 4th month of her first pregnancy this woman developed fever chills, pains in the right flank, anorexia and vomiting, followed by oliguria In the 5th month, while fever continued, frank jaundice was observed with a large and tender liver Massive pyuria continued Purpura developed on the extremities An induced abortion was performed and the woman recovered rapidly During the 7th month of the second pregnancy there was again pyuria, dysuria, pollacisuria and finally oliguria, shortly followed by jaundice with a large tender liver This time there was no temperature elevation The woman delivered 2 weeks later and jaundice disappeared rapidly

Since the advent of sulfonamides and antibiotics no similar cases have been observed

Recurrent jaundice during pregnancy due to hyperemesis gravidarum

A well documented case of this disorder is presented by Thorling (case 33) This woman had hyperemesis during her first pregnancy without jaundice and developed pruritus during the last month of gestation During the second pregnancy she had again marked hyperemesis starting in the 11th week, followed shortly by itching dark urine and manifest jaundice There was urobilinuria no bilirubinuria, an increase in serum bilirubin and alkaline phosphatase and a normal thymol turbidity Upon hospitalisation

the syn ptoms subs ded rap dl) and she deli ered thout further compl cations at term. During the next pregnancy she had aga n l yperemesis tl s t me last ng during her hole gestation ith nter m ttent ep sodes of dark urine and jaun dice Pruritus de eloped during the last two months Upon delivery she became immed ately symptomfree

Ano her poss ble example is the case reported b Lantucjoul and Chambraud This oman had hepat t s at 12 years of age During her first t o pregnancies she suffered from marked hyperemes s w hout jaundice and deli ered prema turel bo l t mes The sequence of e ents was tl e same n her th rd pregnancy but th s t me dark urine and jaundice ere noted before delivery n the th mo th Dur ng the 4th pregnancy hyper emes s started in the 6th month and jaund ce n the 7th Bilirub n and uro bilin ere present in the urine serum b lirub n rose to 5 7 mg per 100 ml the th mol turb d ty and the cephalin floc culat o ere positive. After del ery a fe eeks later she rap dly reco ered

Tl ere are no reported cases th de ta led laboratory data and in none has a l er b ops been performed (see also page 3 Jaund ce due to hype emes s s clea l d stinct from ntrahepat c cl olestas s of pregnancy by tl e pre ence of om t ng and by ts cure well before del ery

Re nt jaund ce du ng p egnancy w tl d ffe ent et ology of jaund ce n u c e pregnanc e

Tl ree case r ports w ll illustrate the diag ost c d ff cult es encountered in s me cases of recurrent jaund ce during pregnancy In these jaund ce of dif ferent origin occurred in successive preg nanc es or jaundice was of mixed etio logy

Case 16 reported by Nixon et al had an abortion during the 6th w eek of her first gestation In the 20th w eek of her second pregnancy she developed ano- rexia nausea and vomiting then dark urine and l ght stools follo ed by frank jaundice in the 24th week Liver and spleen were enlarged serum b lirubin rose to 18 2 mg per 100 ml and l ver biopsy revealed typ cal acute viral hepa titis She del vered in the 30th eek and jaund ce cleared only w thin 2—3 months. Five months later a li er b op sy as normal One and a half years later she was again pregnant During the 5th month she became icteric. The total serum bilirubin was 3 4 mg per 100 ml with normal amounts of the di rect reacting fraction and hemoglobin dropped to 45 % In the 7th month total bil rubin was 1 4 mg per 100 ml While this patient had proved v ral hepatit s during the second pregnancy it appears probable that she had hemolytic jaund ce during the third

Less clear is a case reported by Eyes and Bourdon This woman was jaun d ced from the 5tl month unt l term in l er f rst t o pregnanc es and jaund ce cleared rap dly after del ery Jaund ce again became apparent in the 6th month of her 3rd pregnancy Exam nations at that time revealed a total serum b l rub n of 9 4 mg per 100 ml (d rect reacting 6 4 mg per 100 ml) a lo se rum cholesterol a prolonged prothrom b n time and an ncrease of gamma globul ns to 33 % on electrophores s.

She had a hematocrit of 36 % and a reticulocyte count of 6 %. Here, hemolysis appears to be an additional factor to whatever was the primary disease, which could have been chronic hepatitis or cirrhosis of the liver.

A similar case has been observed by Lebon et al. During the first two pregnancies this woman was jaundiced from the 7th month until shortly after her deliveries in the 8th month. During her third pregnancy she developed asthenia and pruritus in the 5th month, followed by intense arthralgias, back and chest pains, and then by jaundice. The liver was enlarged and tender, her general health markedly impaired, with a weight loss of 13 kg. Bilirubinuria was present, total serum bilirubin was 2.4 mg per 100 ml, alkaline phosphatase 15.8 Bodansky units, the flocculation reactions normal, serum cholesterol low, and liver biopsy showed a granular degeneration of the liver cells, an increase in Kupffer cells and an increase in reticulum. This was interpreted as "periportal hepatitis". Her red cell count was 2 700 000 per cu mm and reticulocytes were 3.4 %, so that hemolysis appears superimposed to what was — from the history — viral hepatitis during her 3rd pregnancy. The episodes of jaundice during her first two pregnancies are not explained. Hypothetically it is possible that a woman has recurrent intrahepatic cholestasis during two pregnancies and then viral hepatitis during the third.

Recurrent jaundice during pregnancy due to unclassified causes

Five cases of recurrent jaundice during pregnancy cannot be classified at all.

Two sisters with recurrent jaundice during pregnancy were reported by both Benedict and Lovrich. Pruritus and jaundice occurred in case 1 during 4, in case 2 during 2 gestations. In both cases onset of jaundice was in the first trimester. During the fourth pregnancy of case 1 the liver and the spleen were markedly enlarged and hard, during the second pregnancy of case 2 the liver was enlarged and the spleen was not palpable but enlarged to percussion. Cirrhosis of the liver or a familial hemolytic disorder (urine constantly negative for bilirubin) are not excluded in these cases.

The case described by Vignes in 1937 with jaundice during the 7th to 10th pregnancies has been considered to represent hemolytic jaundice by the author. The patient had tachycardia, dyspnoe, oliguria and pruritus besides jaundice, and looked "increasingly toxic". Not enough details are given to permit a retrospective evaluation.

Case 19 of Nixon et al. was jaundiced from the 24th week until delivery at term in her first pregnancy. Jaundice appeared again in the 10th week of her second pregnancy. The liver was enlarged and total serum bilirubin rose to 10.5 mg per 100 ml. Two liver biopsies were performed, both showing hyperplasia of the Kupffer cells and a fair amount of biliary pigment in the reticulum cells. The patient was not followed. In this case a hemolytic disorder or a subsiding viral hepatitis may be considered.

Case II of Boquien et al. was jaundiced during the 2nd, 3rd and 4th pregnancy. During the second pregnancy

eclampsia with hypertension and proteinuria occurred during the 8th month, followed by jaundice, convulsions, and delivery of a stillborn child by Caesarian section During the 8th month of the 3rd pregnancy hypertension was observed and then delivery of a stillborn child took place in the presence of a retroplacentar hematoma Jaundice began at delivery and lasted for some weeks Hemolysis was excluded During the fourth pregnancy — again complicated by hypertension — pruritus and jaundice began one day *after* Caesarian section in the 8th month lasting for 10 days As jaundice began *after* delivery in the last two gestations intrahepatic cholestasis of pregnancy seems excluded Eclampsia appears to have caused jaundice during the second and possibly also during the 3rd pregnancy

The case reported by Justin—Besançon et al had onset of pruritus and jaundice during the first month of her first pregnancy associated with vomiting She delivered at term and jaundice disappeared two weeks later General health was markedly impaired and she lost 10 kg body weight during her gestation During her second gestation jaundice and pruritus appeared in the 7th week after a prodromal phase of anorexia and vomiting Because of impaired general condition and weight loss of 5 kg abortion was induced in the 12th week This resulted in fever up to 38° centigrade and in an increase in jaundice Twelve days later her serum bilirubin was 18 0 mg per 100 ml Three weeks after delivery serum bilirubin was 3 5 mg per 100 ml with positive reactions to the thymol turbidity and the zinc sulfate flocculation Peritoneoscopy on the 31st day after delivery was unremarkable, bromsulfalein retention on the 37th day was 45 % after 45 minutes, a liver biopsy on the 43rd day (2 weeks after subsidence of jaundice) showed minimal cholestasis, and radiological gallbladder examination was normal The general symptoms and weight loss, as well as the intensity of jaundice would point towards viral hepatitis as the cause of jaundice during the second gestation, but this cannot be conclusively proved

Misquoted cases of recurrent jaundice during pregnancy in the literature

The case of Bjerregaard, published in 1904, had 3 normal pregnancies, then three with pruritus alone, followed by pruritus and jaundice during the 7th gestation only This case is repeatedly cited as representing recurrent jaundice during pregnancy In these quotations the author s name is usually mis spelled and the bibliography incomplete or incorrect Most likely few reviewers bothered to have the Danish original translated

Another case often quoted is one published in a French journal in 1907 by M L A Meyer The case report is truly an example of recurrent jaundice during pregnancy but represents only a French abstract of a German paper by A Mayer in 1906 with the author's name mis spelled Among the author s initials in the French abstract the M stands for monsieur and the origin of the L cannot be traced.

A case of Enrile et al with tuberculoma of the liver has been called recurrent jaundice occasionally This

She had a hematocrit of 36 % and a reticulocyte count of 6 %. Here, hemolysis appears to be an additional factor to whatever was the primary disease, which could have been chronic hepatitis or cirrhosis of the liver.

A similar case has been observed by Lebon et al. During the first two pregnancies this woman was jaundiced from the 7th month until shortly after her deliveries in the 8th month. During her third pregnancy she developed asthenia and pruritus in the 5th month, followed by intense arthralgias, back and chest pains, and then by jaundice. The liver was enlarged and tender, her general health markedly impaired, with a weight loss of 13 kg. Bilirubinuria was present, total serum bilirubin was 2.4 mg per 100 ml, alkaline phosphatase 15.8 Bodansky units, the flocculation reactions normal, serum cholesterol low, and liver biopsy showed a granular degeneration of the liver cells, an increase in Kupffer cells and an increase in reticulum. This was interpreted as "periportal hepatitis". Her red cell count was 2,700 000 per cu mm and reticulocytes were 3.4 %, so that hemolysis appears superimposed to what was — from the history — viral hepatitis during her 3rd pregnancy. The episodes of jaundice during her first two pregnancies are not explained. Hypothetically it is possible that a woman has recurrent intrahepatic cholestasis during two pregnancies and then viral hepatitis during the third.

Recurrent jaundice during pregnancy due to unclassified causes

Five cases of recurrent jaundice during pregnancy cannot be classified at all.

Two sisters with recurrent jaundice during pregnancy were reported by both Benedict and Lovrich. Pruritus and jaundice occurred in case 1 during 4, in case 2 during 2 gestations. In both cases onset of jaundice was in the first trimester. During the fourth pregnancy of case 1 the liver and the spleen were markedly enlarged and hard, during the second pregnancy of case 2 the liver was enlarged and the spleen was not palpable but enlarged to percussion. Cirrhosis of the liver or a familial hemolytic disorder (urine constantly negative for bilirubin) are not excluded in these cases.

The case described by Vignes in 1935 with jaundice during the 7th to 10th pregnancies has been considered to represent hemolytic jaundice by the author. The patient had tachycardia, dyspnoe, oliguria and pruritus besides jaundice, and looked increasingly toxic. Not enough details are given to permit a retrospective evaluation.

Case 19 of Nixon et al. was jaundiced from the 24th week until delivery at term in her first pregnancy. Jaundice appeared again in the 10th week of her second pregnancy. The liver was enlarged and total serum bilirubin rose to 10.5 mg per 100 ml. Two liver biopsies were performed, both showing hyperplasia of the Kupffer cells and a fair amount of biliary pigment in the reticulum cells. The patient was not followed. In this case a hemolytic disorder or a subsiding viral hepatitis may be considered.

Case II of Boquien et al. was jaundiced during the 2nd, 3rd and 4th pregnancy. During the second pregnancy

eclampsia with *hypertension and* protein uria occurred during the 8th month, followed by jaundice, convulsions, and deli*very* of a stillborn child by Caesarian section During the 8th month of the 3rd pregnancy hypertension was observed and then delivery of a stillborn child took place in the presence of a *retroplacentar* hematoma Jaundice began at delivery and lasted for some weeks Hemolysis was excluded During the fourth pregnancy — again compli cated by hypertension — pruritus and jaundice began one day after Caesarian section in the 8th month lasting for 10 days As jaundice began *after* delivery in the last two gestations, intrahepatic cholestasis of pregnancy seems excluded Eclampsia appears to have caused jaundice *during the second and possibly also* during the 3rd pregnancy

The case reported by Justin—Besan çon et al had onset of pruritus and jaun dice during the first month of her first pregnancy associated with vomiting She delivered at term and jaundice dis appeared two weeks later General health was markedly impaired and she lost 10 kg body weight during her gesta tion During her second gestation jaun dice and pruritus appeared in the 7th week after a prodromal phase of ano*rexia and vomiting* Because of impaired general condition and weight loss of 5 kg abortion was induced in the 12th week This resulted in fever up to 38° centigrade and in an increase in jaun dice Twelve days later her serum bili rubin was 18 0 mg per 100 ml Three weeks after delivery serum bilirubin was 3 5 mg per 100 ml with positive reac tions to the thymol turbidity and the zinc sulfate flocculation Peritoneoscopy on the 31st day after delivery was un remarkable, bromsulfalein retention on the 37th day was 45 % after 45 minutes, a liver biopsy on the 43rd day (2 weeks after subsidence of jaundice) showed minimal cholestasis, and radiological gallbladder examination was normal The general symptoms and weight loss, as well as the intensity of jaundice would point towards viral hepatitis as the cause of jaundice during the second gestation, but this cannot be conclusively proved

Misquoted cases of recurrent jaundice during pregnancy in the literature

The case of Bjerregaard, published in 1904, had 3 normal pregnancies, then three with pruritus alone, followed by *pruritus and jaundice during the 7th* gestation only This case is repeatedly cited as representing recurrent jaundice during pregnancy In these quotations the author's name is usually mis spelled and the bibliography incomplete or in correct Most likely few reviewers both ered to have the Danish original trans lated

Another case often quoted is one pub lished in a French journal in 1907 by M L A Meyer The case report is truly an example of recurrent jaundice during pregnancy but represents only a French abstract of a German paper by A Mayer in 1906 with the author's *name mis spelled Among* the author s initials in the French abstract the M stands for monsieur and the origin of the L cannot be traced

A case of Enrile et al with tuber culoma of the liver has been called re current jaundice occasionally This

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Case II of Boquien et al. was jaundiced during the 2nd, 3rd and 4th pregnancy. During the second pregnancy

plete recovery in the anicteric intervals It shall be briefly mentioned, mainly to show that its course differs markedly in all other respects from that seen in intrahepatic cholestasis of pregnancy An other probably non-existing entity referred to in the older literature shall be listed to complete this survey

Idiopathic recurrent cholestasis

Of this curious and only recently discovered disease only 12 cases have so far been described (Summerskill and Walshe 1959 2 cases, Tygstrup 1960 2 cases De Groote et al 1960 2 cases, Kühn 1960 2 cases Schapiro and Isselbacher 1963 1 case and Williams et al 1964 3 cases) The entity consists of recurrent episodes of intrahepatic obstructive jaundice with complete clinical functional and histological recovery in the intervals Two patients are females and 10 are males Two patients are brothers (Kuhn) two adolescent males are possibly related (Tygstrup) and in the other eight the family history is negative First symptoms occurred between the ages of 1 and 29 years with 5 patients in the first 4 in the second and 3 in the third decade The longest observed duration after the first attack of jaundice is 37 years with 27 episodes of jaundice recorded (Schapiro and Isselbacher)

An attack starts usually with a mild prodromal syndrome of anorexia and fatigue of up to 2 weeks duration Marked pruritus and jaundice then set in lasting usually around 4 months with a range from 1 to 10 months There is no fever or pain Steatorrhea (up to 48 gm fecal fat excretion per day (Tygstrup case 2)) during jaundice leads to a marked weight loss, despite good appetite Weight loss is always regained in the asymptomatic interval Jaundice is usually accompanied by moderate hepatomegaly The spleen was slightly enlarged in two cases Chemically, an obstructive pattern is observed Serum bilirubin levels are usually between 8 and 23 mg per 100 ml, but may reach 40 mg per 100 ml Serum alkaline phosphatase is mildly to moderately elevated, but may attain 94 King—Armstrong units Serum cholesterol, however, is usually normal It was mildly elevated in 2 cases (Tygstrup) and reached 380 mg per 100 ml in the case which also presents the highest recorded levels for serum bilirubin and alkaline phosphatase (Schapiro and Isselbacher) Serum transaminases have been normal or only slightly elevated Serum turbidity and flocculation tests are normal as well as the albumin/globulin ratio The erythrocyte sedimentation rate is slightly elevated and electrophoresis shows a mild increase in alpha 2 and beta globulins (Kuhn, Williams et al) Intravenous bilirubin tolerance tests show a regurgitation of conjugated bilirubin from the liver cells into the blood stream during jaundice but are normal in the anicteric interval (Williams et al) Gallbladder X rays usually show no filling during jaundice but all intraoperative cholangiograms have been normal

Liver biopsies during jaundice show marked centrolobular bile stasis with bile plugs in the canaliculi and bile pigment in the hepatocytes and in the Kupffer cells In the centrolobular areas there is hepatocellular degeneration loss of liver cells and inflammatory infiltra

Table 23 Recurrent jaundice *during* pregnancy

A Recurrent jaundice *in* pregnancy

I Recurrent jaundice recurring also in non pregnant subjects
- 1 Recurrent viral hepatitis or exacerbation of anicteric chronic hepatitis
- 2 Recurrent jaundice in primary biliary cirrhosis
- 3 Recurrent posthepatitic hyperbilirubinemia
- 4 Recurrent common bile duct obstruction due to gall stones
- 5 Recurrent exacerbations of familial non hemolytic jaundice
- 6 Recurrent hemolytic jaundice
- 7 Recurrent jaundice with hemoglobinuria (??)

II Recurrent jaundice due to medical complications of pregnancy
- 1 Recurrent jaundice in severe pyelonephritis

B Recurrent jaundice *of* pregnancy

I Recurrent idiopathic jaundice of pregnancy
- 1 Recurrent intrahepatic cholestasis of pregnancy

II Recurrent jaundice as complication of disease linked to pregnancy
- 1 Recurrent jaundice in hyperemesis gravidarum

C Recurrent jaundice during pregnancy due to different diseases causing jaundice during pregnancy
Example Hepatitis in one hemolytic jaundice in the other gestation

D Non classified cases of recurrent jaundice during pregnancy

woman had 3 episodes of jaundice during a single pregnancy (her fifth)

4) Classification of recurrent jaundice during pregnancy

A classification of recurrent jaundice during pregnancy based on the cases described in chapter III/2 and III/3 is given in Table 23 The same general subdivisions are used as in Table 3 in the classification of (non recurrent) jaundice during pregnancy It is theoretically possible, that other diseases listed in Table 3 and not listed in Table 23 could be recurrent, such as jaundice due to toxemia of pregnancy or jaundice due to tetracycline toxicity We have refrained from listing them under recurrent jaundice during pregnancy before actual cases with adequate documentation are presented

5) Other diseases with recurrent jaundice and complete recovery in the anicteric interval

Many patients with chronic liver disease such as chronic hepatitis or cirrhosis of the liver may run a course characterized by periods of exacerbations with jaundice and relatively symptom free anicteric intervals Residual functional and/or structural damage persists, however, in these diseases Apart from recurrent intrahepatic cholestasis of pregnancy only one disease is at present known with recurrent jaundice and com

dice (Kehrer 1905), but some still denied a connection vehemently (Schickele 1910) or believed these cases to be due to gallstone obstruction (Rissmann 1910)

Gallstone obstruction of the common bile duct as a cause of cholestasis of pregnancy has been excluded since radiological examination of the gall bladder has been possible Ikonen considers the incidence of *coincidental* gall stones not causing obstruction high in intrahepatic cholestasis of pregnancy (this opinion is based on non recurrent cases) and believes that gall stone formation could well be explained as a parallel phenomenon arising from the same disorder in metabolism which is causing the clinical picture of obstetric hepatosis

With the recognition of viral hepatitis as one of the most common liver diseases during World War II the causal relationship between pregnancy and recurrent jaundice was questioned again and many especially French authors considered the disorder to represent a *recurrence or exacerbation of a previous* viral hepatitis under the stress of pregnancy The arguments for or against such a viewpoint have been summarized by Caroli et al in 1954 who concluded that recurrent jaundice of pregnancy will but rarely be caused by viral hepatitis

A type of latent familial non hemolytic jaundice with manifestation during pregnancy is suggested by the familial occurrence of intrahepatic cholestasis of pregnancy in at least 6 instances (see page 70 Gilbert's syndrome with an elevation of the indirect serum bilirubin fraction only is ruled out immediately Both the Rotor and the Dubin– Johnson syndrome have an elevation of the direct reacting bilirubin fraction In the Dubin—Johnson syndrome pruritus is usually absent and the alkaline phosphatase not elevated Liver biopsy with the characteristic blackbrown pigment is quite different from liver biopsy in intrahepatic cholestasis of pregnancy (Dubin) Liver biopsy in the Rotor syndrome is normal, as it is in some cases of intrahepatic cholestasis of pregnancy Laboratory findings are somewhat similar, but bromsulfalein retention is more pronounced and oral contrast materials visualize the gallbladder well The patients with the Rotor syndrome are asymptomatic and do not have pruritus (Schiff et al , Peck et al) Furthermore jaundice decreases during pregnancy (Haverback and Wirtschafter)

The concept of bile stasis was probably first advanced by Boreel in 1924 It was clearly expressed by Perreau in 1933 and by Svanborg in 1954 Thorling in 1955 proposed the concept of *incomplete intrahepatic* biliary tract obstruction precipitated by hepatic damage The main theories advanced to explain the mechanism of intrahepatic cholestasis in this disorder are atony of the extrahepatic biliary passages (Svanborg) change in the chemical composition of bile leading to inspissation (Ljunggren) and a change in membrane permeability at the level of the bile capillaries (Gros) The first two possibilities appear unlikely because of the observation of normal bile drainage from a T tube in the case of Beraud et al This provides of course no evidence in

tion Some portal tracts show edema or infiltration (Williams et al) Biopsies in anicteric intervals are normal or may show *minimal* residual bile stasis Laparatomies and peritoneoscopies have revealed a brownish or greenish discoloration of the liver but otherwise normal findings

Both of the 2 reported women have been pregnant during the course of their disease The case with 27 episodes of jaundice during age 3 and 40 underwent a single normal anicteric pregnancy at age 25 (Schapiro and Isselbacher) The other woman had her first episode of jaundice in the second month of her first pregnancy After an artificial abortion in the fourth month of gestation the jaundice persisted Three further pregnancies were anicteric during the whole course

Thus, pregnancy does not induce a relapse of jaundice in this disease

Recurrent jaundice during menstruation

In 1872 Senator described 4 women who developed jaundice for a few days during each successive menstrual bleeding A careful review of his case reports does not support his enthusiastic association of jaundice with menstruation The maximal episodes of jaundice in a single patient were six, with anicteric menstruations or — in one case — a normal pregnancy thereafter Furthermore the time relationship is not always convincing During the 'disease' jaundice is said to occur instead of a missed menstrual bleeding From the data presented it is impossible to diagnose the nature of this jaundice A fifth case in the literature, reported by Metzger in 1904, is clearly a description of an acute viral hepatitis with several *relapses*

No further such cases have been described and it must be concluded that 'recurrent jaundice during menstruation" does not exist

6) Pathogenesis of intrahepatic cholestasis of pregnancy

A review of the older literature concerning speculations on the pathogenesis of recurrent jaundice during pregnancy has been presented by Mayer in 1906 The prevalent idea at that time was a mechanical compression of the extra hepatic bile ducts by the enlarged uterus pressing on a constipated transverse colon This mirrors the views held by the authors of textbooks on liver disease in the 19th century (Budd and Henoch 1846, Frerichs 1858, Quincke and Hoppe—Seyler 1899) Other theories on the pathogenesis of benign jaundice during pregnancy included "nervous influences", 'plethora of organs during pregnancy', "dyspepsia', "gastroduodenitis", a 'mucous plug in the common bile duct' and "sudden emotions' (Meunier 1872)

A similar line of thought is persued by Roumanian authors in 1957 (Pavel et al) who assume that distension of the peritoneum overlying the enlarging uterus triggers nervous reflexes which then will — modified by a terrain of hyperfolliculinemia — lead to a spasm of the sphincter of Oddi and perhaps to reflex inhibition of bile secretion

Early in the 20th century most authors were convinced of a causal relationship between pregnancy and recurrent jaun

dice (Kehrer 1903), but some still denied a connection vehemently (Schickele 1910) or believed these cases to be due to gallstone obstruction (Rissmann 1910)

Gallstone obstruction of the common bile duct as a cause of cholestasis of pregnancy has been excluded since radiological examination of the gall bladder has been possible Ikonen considers the incidence of coincidental gall stones not causing obstruction high in intrahepatic cholestasis of pregnancy (this opinion is based on non recurrent cases) and believes that gall stone formation could well be explained as a parallel phenomenon arising from the same disorder in metabolism which is causing the clinical picture of obstetric hepatosis

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favour of the membrane permeability theory

Whenever the etiology of a disease is unknown, one is inclined to draw analogies to similar diseases. The syndrome intrahepatic cholestasis includes (apart from pregnancy) viral hepatitis, drug induced cholestasis, acute fatty liver and alcoholism (Dolle and Martini 1959 and 1962, Jeffries and Sleisenger).

Fatty liver and alcoholism can readily be excluded on the basis of liver biopsy findings and personal history in patients with intrahepatic cholestasis of pregnancy.

Viral hepatitis may occur under the form of pure intrahepatic cholestasis without signs of inflammation or liver cell damage on liver biopsy (Caroli et al 1953, Dubin et al 1960, histological type D). These cases are extremely rare (6 patients in these two papers) and present clinically the usual prodromal symptoms seen in typical acute viral hepatitis, which are absent in intrahepatic cholestasis of pregnancy. More frequent are forms of viral hepatitis with obstructive features (so-called cholangiolitic hepatitis) with findings of typical viral hepatitis on biopsy (Watson and Hoffbauer, Gall and Braunstein, Dubin et al, histological types B and C) so that they are easily distinguishable from intrahepatic cholestasis of pregnancy.

Primary biliary cirrhosis may in the initial stages present with pruritus without jaundice, with an elevation of alkaline phosphatase and an elevation of bromsulfalein retention (Popper et al 1962). No case of recurrent cholestasis of pregnancy has yet progressed to primary biliary cirrhosis. The case reported by Tylecote probably represented primary biliary cirrhosis from the onset of its clinical course.

Drug induced cholestasis is most likely to induce comparison with intrahepatic cholestasis of pregnancy, especially as sex hormones can be implicated in both disorders. In addition Read et al suggested that pregnant women may be especially susceptible to the development of chlorpromazine induced jaundice, although this has not been confirmed by others (see page 32).

Drug induced intrahepatic cholestasis consists of two main types: a sensitivity type (example chlorpromazine jaundice) which histologically shows portal infiltrations in addition to cholestasis, and a non sensitivity type (example norethandrolone jaundice) with the histological picture of pure cholestasis (Sherlock). The latter type is histologically comparable to intrahepatic cholestasis of pregnancy. It is produced by methyl testosterone and other C 17 α alkyl substituted testosterones such as norethandrolone (Nilevar®), methandienone (Dianabol®) and the ovulation inhibitor Enavid®, which contains norethynodrel.

In this connection an interesting observation has been made in case L. A. reported by Ikonen. This woman with intrahepatic cholestasis of pregnancy in 2 successive gestations was given an ovulation inhibitor containing norethisterone and aethinyloestradiol 9 1/2 months after the last delivery. She developed abdominal pains, nausea and — after one week — dark urine and pruritus, followed by jaundice and an increase in

SGOT The drug was stopped and jaundice disappeared The patient later received progesterone without ill effect

King and Kerrins, in their report on a case with recurrent intrahepatic cholestasis of pregnancy, considered this disorder to have a "striking resemblance to drug induced jaundice" Similar opinions have been voiced by Svanborg and Ohlsson and by Gros Such statements have to be firmly refused Although the case of Ikonen suggests that pregnancy and the ovulation inhibitor triggered the same disease process and although histology in norethandrolone-type cholestasis is indistinguishable from intrahepatic cholestasis of pregnancy, there are important and mutually exclusive differences between the two disorders Jaundice in intrahepatic cholestasis of pregnancy is always mild and is not known to have surpassed a serum bilirubin level of 8 ½ mg per 100 ml In drug induced cholestasis jaundice is mild in the majority of cases, but may reach levels of up to 30 mg per 100 ml of serum bilirubin (Werner et al) In norethandrolone type jaundice alkaline phosphatase is comparatively little elevated Furthermore a prodromal period with anorexia malaise and often fever is common in drug induced jaundice (Schaffner) and was present in the ovulation inhibitor induced jaundice in Ikonen s case while prodromi are absent in intrahepatic cholestasis of pregnancy In addition pruritus is the dominant symptom in intrahepatic cholestasis of pregnancy while in drug induced jaundice pruritus is present in only about half the cases More significant even, methyl testosterone and norethandrolone are effective therapeutic agents to relieve pruritus of hepatic origin, although they increase the intensity of pre existing jaundice at the same time.

A disordered hormonal balance remains an attractive hypothesis for the basic pathogenetic mechanism in intrahepatic cholestasis of pregnancy, but such a hypothesis should not be founded on a comparison of this disease with steroid induced jaundice as we know it at present

It might be worth while to consider briefly the meaning of the term "intrahepatic cholestasis" Cholestasis has been given two basic definitions a clinical-functional one and a histological one. Clinically and functionally cholestasis is defined as a disease with pruritus, an elevation of serum alkaline phosphatase and often an elevation of serum cholesterol Histologically cholestasis is defined as the presence of bile plugs (or bile thrombi) in the bile canaliculi (or bile capillaries), and the presence of bile pigment in hepatic cells and Kupffer cells predominantly in the centrolobular area (Popper and Schaffner) While in most examples of intrahepatic cholestasis functional and histological cholestasis are present more or less parallel to one another in others they are not and functional or histological cholestasis may be completely absent On one end of this spectrum lies the entity called "post operative intrahepatic cholestasis" which presents a marked and pure cholestasis on liver biopsies but functionally predominantly an elevation of serum bilirubin of up to 27 mg per 100 ml with normal or mildly elevated alkaline phosphatase and absence of pruritus (Schmid

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12 Drainage of bile from a T tube in the common bile duct is normal during jaundice

13 Premature delivery is frequent, but limited to some women, while about two thirds of the patients deliver at term. There is no correlation between premature delivery and any other feature of the disorder

14 The intensity of symptoms during successive pregnancies with jaundice may remain equal, may increase or may decrease

15 Recurrent pruritus gravidarum may be observed before recurrent pruritus with jaundice in some cases

At present no pathogenetic explanation based on facts can be offered at least none giving an insight into what ever process launches the disorder It appears reasonable however to assume that — whatever this triggering process is — *physiological derangement of liver function in normal pregnancy pruritus gravidarum and intrahepatic cholestasis of pregnancy are but three increasing grades of manifestation of the same basic disorder linked in some way to pregnancy* (Friedberg 1951)

In uncomplicated pregnancies there is towards term an increase in alkaline phosphatase, *serum cholesterol*, *serum* lipids alpha and beta globulins and bromsulfalein retention, and a decrease in bromsulfalein excretory capacity (BSB T_m) and intravenous bilirubin tolerance In pruritus gravidarum these same alterations are more pronounced *and additionally the serum transaminases* are mildly elevated Intrahepatic cholestasis of pregnancy shows again an increase of the disturbances seen in pruritus gravidarum with the addition of an elevated serum bilirubin, a radiologically non visualized gallbladder and histological evidence of intrahepatic cholestasis

Thus intrahepatic cholestasis of pregnancy is best viewed as an exaggeration of the alterations of liver function occurring to a minor degree also in uncomplicated pregnancies

et al) Intrahepatic cholestasis of pregnancy represents the other end of this spectrum, as in this disorder pruritus is violent, biochemical cholestasis usually impressive and histological cholestasis focal and minimal, so minimal that it can be easily missed by the pathologist At present no explanation can be offered for this divergence of functional and structural cholestasis, but this aspect should be kept in mind when pathogenesis is discussed

An explanation of the pathogenesis of recurrent intrahepatic cholestasis of pregnancy has — in order to be of value — to explain all of the following features in this disorder

1 The disorder is strictly linked to pregnancy and does not occur in non pregnant subjects
2 The disorder usually presents as 'jaundice during late pregnancy" although onset of jaundice may be as early as in the first trimester in some cases
3 Pruritus is the first symptom to occur and the main symptom during the course of the disease
4 Apart from pruritus there are no prodromal symptoms There is a conspicuous absence of general symptoms such as fever, weakness, malaise, anorexia, nausea, vomiting, dyspepsia, pains, colics, arthralgias, weight loss
5 Jaundice is always of mild degree The highest observed serum bilirubin level is 8 4 mg per 100 ml In most cases it is below 6 mg per 100 ml Direct reacting bilirubin constitutes the main portion of total serum bilirubin *elevation Bilirubinuria* is transient or intermittent, but probably present in all cases
6 Serum alkaline phosphatase and serum cholesterol are elevated in most and serum transaminases in many cases Any of these three parameters may occasionally be normal in a single patient. Highest observed values are alkaline phosphatase 28 Bodansky units, serum cholesterol 590 mg per 100 ml, SGOT 920 units, SGPT 875 units (although transaminases are with few exception below 250 units) Serum electrophoresis shows an increase in alpha 2- and mainly in beta globulins, with normal or slightly decreased gamma-globulins There is no correlation between any two laboratory tests
7 Bromsulfalein retention is increased and galactose tolerance is normal Bromsulfalein clearance studies reveal a decreased hepatic storage capacity and an increased cholestatic index
8 Rapid improvement sets in after delivery, whether delivery is spontaneous or induced
9 In closely followed cases there may be a transient return to normal of serum bilirubin and of transaminases on the first postpartum day
10 Recovery after delivery is complete No permanent liver damage ensues after multiple pregnancies with jaundice
11 Histological cholestasis on liver biopsy is mild and focal or irregular This is in marked contrast to the impressive clinical and biochemical *signs of cholestasis*

12 Drainage of bile from a T tube in the common bile duct is normal during jaundice.

13 Premature delivery is frequent, but limited to some women, while about two thirds of the patients deliver at term There is no correlation between premature delivery and any other feature of the disorder

14 The intensity of symptoms during successive pregnancies with jaundice may remain equal, may increase or may decrease

15 Recurrent pruritus gravidarum may be observed before recurrent pruritus with jaundice in some cases

At present no pathogenetic explanation based on facts can be offered at least none giving an insight into whatever process launches the disorder It appears reasonable however to assume that — whatever this triggering process is — *physiological derangement of liver function in normal pregnancy pruritus gravidarum and intrahepatic cholestasis of pregnancy are but three increasing grades of manifestation of the same basic disorder linked in some way to pregnancy* (Friedberg 1951)

In uncomplicated pregnancies there is towards term an increase in alkaline phosphatase, serum cholesterol, serum lipids, alpha- and beta globulins and bromsulfalein retention, and a decrease in bromsulfalein excretory capacity (BSB T_m) and intravenous bilirubin tolerance In pruritus gravidarum these same alterations are more pronounced and additionally the serum transaminases are mildly elevated Intrahepatic cholestasis of pregnancy shows again an increase of the disturbances seen in pruritus gravidarum with the addition of an elevated serum bilirubin a radiologically non visualized gallbladder and histological evidence of intrahepatic cholestasis

Thus intrahepatic cholestasis of pregnancy is best viewed as an exaggeration of the alterations of liver function occurring to a minor degree also in uncomplicated pregnancies

SUMMARY

The main purpose of this review is to define the entity called "recurrent intrahepatic cholestasis of pregnancy" and to establish a differential diagnosis on the many disorders which may present as recurrent jaundice during pregnancy

PART I contains a review on "liver function" during uncomplicated pregnancy The liver performs its function well during gestation, but most so called "liver function tests" show some minor deviations from the accepted normal in non-pregnant subjects These "physiological" derangements are more common in the later weeks of pregnancy and are rapidly rectified after delivery

Liver biopsy findings remain generally within normal limits Some minor nonspecific changes may occur, such as a difference in size of liver cells, an increase in size of their nuclei some irregularities of the nuclei, an increase in bi nucleated cells an increased glycogen content of the cytoplasm and occasionally mild lymphocytic infiltrations in the portal tracts

Spider angiomas and palmar erythema may be found in up to two thirds of pregnant females These skin changes are rarely impressive

Liver blood flow remains quantitatively unchanged As plasma volume and total blood volume increase by about 50 % in normal pregnancy relative liver blood flow (as fraction of cardiac output) decreases somewhat

Hemoglobin falls as a result of increasing plasma volume. Serum iron fluctuates, but remains on the whole constant Total white cell counts may be increased to up to 15,000 per cu mm and a "shift to the left" is not uncommon

Alkaline phosphatase increases towards term, with usually a pronounced rise after the 7th month of gestation Serum cholesterol and serum lipids follow the same trend On electrophoresis a slight increase in alpha 2 and beta globulins is noted These changes, though mild, are indicative of an "obstructive pattern" even in normal pregnancy

Serum bilirubin may in a rare case be elevated to up to 2 mg per 100 ml and abnormal urinary bile pigments may be occasionally present These changes are not related to the stage of pregnancy in contrast to the "obstructive features which tend to increase towards term

Total serum proteins and serum albumin decrease towards term The flocculation and turbidity tests are usually normal, but positive results are seen in varying proportions of cases probably dependent more on the technique used in a specific laboratory than on the test itself

Bromsulfalein retention is slightly in-

creased towards term, due to an increase in hepatic storage capacity and a decrease in maximal excretory capacity Galactose tolerance remains normal

The only "liver function tests" to remain normal throughout pregnancy are the serum transaminases and the prothrombin time

With the exception of the changes in the white cell count most deviations from the normal are minor Main diagnostic difficulties will be encountered with the alkaline phosphatase and the turbidity or flocculation tests

PART II contains a review on all diseases causing jaundice *during* pregnancy Jaundice occurs in about 1 out of every 1 500 gestations, an incidence of 0 067 % At least 41 % of all cases with jaundice are due to viral hepatitis, and about 21 % due to intrahepatic cholestasis of pregnancy Common bile duct obstruction accounts for less than 6 % of all cases with jaundice

A classification of jaundice *during* pregnancy is proposed in Table 3

A first group of diseases "jaundice *in* pregnancy" consists of entities seen also in non pregnant persons occurring by chance during gestation

Viral hepatitis during pregnancy has often been quoted to run a severe cause during pregnancy A review of the literature reveals that pregnant women are not more susceptable to viral hepatitis than non pregnant subjects that viral hepatitis runs the same course *in* pregnancy as outside of it and that mortality from viral hepatitis is not increased during pregnancy at least not in Europe An exception to this rule may be seen in the indigene population of underdeveloped countries, where malnutrition and general debility provide a serious hazard to any additional injury during gestation Viral hepatitis occurs probably with equal frequency during all trimesters of gestation and not predominantly towards term, as has been frequently stated The course of severe hepatitis is not influenced by an induced interruption, and termination of pregnancy is not advised Hepatitis induces an increased incidence of premature deliveries Child survival depends on the degree of maturity No conclusive evidence exists that viral hepatitis can be transmitted from the mother to the unborn child at least not in the later part of gestation

Pregnancy occurs rarely during cirrhosis of the liver because fertilization is impaired in this disease For this reason, cirrhotics who do get pregnant probably represent a selection of the less severe cases and generally tolerate gestation surprisingly well even when they previously had manifestations of decompensated liver function Pregnancy increases intra abdominal pressure and probably also pre-existing portal hypertension A rare case will develop ascites Occasionally hematemesis from esophageal varices is induced Successful porto-caval shunts have been performed during pregnancy Hematemesis towards terms may be stopped by a Caesarian section Child survival is not affected by the mother's *cirrhosis*

Drug induced intrahepatic cholestasis (usually due to chlorpromazine) is probably not influenced by pregnancy although 4 of 22 chronic cases in the

literature began in pregnancy, including the case with the longest recorded duration of 3 years before complete recovery. The prodromal phase is similar to that seen in viral hepatitis, the biochemical data show an "obstructive pattern".

Gallstone disease is — contrary to general opinion — in most likelyhood not caused by nor increased during pregnancy and common bile duct obstruction is rare during gestation. It is handled according to general medical or surgical principles.

Data on the behaviour of chronic idiopathic hyperbilirubinemias are sparse. Pregnancy generally aggravates the intensity of jaundice in the Dubin—Johnson syndrome, may lessen it in the Rotor syndrome, and does usually not influence the course in Gilbert's syndrome.

Hemolytic disorders during pregnancy usually present as marked anemia and an accompanying jaundice is generally mild. The primary hemolytic disorders during pregnancy — megaloblastic anemia of pregnancy due to folic acid deficiency and idiopathic hemolysis of pregnancy — are exceedingly rare. Pre existing chronic hemolytic states may be aggravated during gestation, such as familial spherocytosis and some hemoglobinopathies, especially S—C, S—S and C—C disease. The combination of hemoglobinopathies and pregnancy is hazardous for both mother and child. Secondary hemolysis may be observed after incompatable blood transfusions and in overwhelming septicemias.

Jaundice occurring in severe pyelonephritis during pregnancy (probably due to septicemia) has nearly disappeared since the advent of the antibiotics. A new syndrome has replaced it: jaundice in tetracycline toxicity used in the treatment of pyelonephritis during pregnancy. This toxic liver damage is often fatal. Postmortem examination reveals a diffuse fatty metamorphosis of the liver. So far it has not been seen in nonpregnant subjects.

Jaundice due to delayed chloroform poisoning and jaundice in criminal abortions are mainly of historic interest.

A second group of diseases, "jaundice *of* pregnancy", consists of disorders caused by pregnancy itself or occurring in typical complications linked to pregnancy.

Intrahepatic cholestasis of pregnancy is an entity benign to both mother and child, but has a marked tendency to recur in successive gestations. There are no prodromal symptoms such as in viral hepatitis or as in drug induced intrahepatic cholestasis. The disease is characterized by often violent pruritus and mild jaundice, both disappearing usually within two weeks after spontaneous or induced delivery. Onset of jaundice is generally after the 22nd week of gestation but may occur as early as the 7th week. Biochemically there is an obstructive pattern with an increase of serum bilirubin, alkaline phosphatase, cholesterol, and alpha 2 and beta globulins on electrophoresis. Transaminases are mildly to moderately increased. Abnormal bromsulfalein retention is present but galactose tolerance is normal. Flocculation and turbidity reactions are normal. Radiological gallbladder examinations show no filling during jaundice but bile flow in the extrahepatic biliary

system is unimpaired. Liver biopsy shows only a mild and usually focal or irregular cholestasis with no evidence of liver cell damage.

Acute fatty metamorphosis of pregnancy is a rare disorder with a very high mortality. Histologically it presents as a diffuse fatty change of the liver with the exception of a sharply defined rim of normal liver cells around the portal tract. There is no necrosis or inflammation. Clinically the disease resembles fulminant viral hepatitis. Onset of symptoms is always after the 30th and in the majority after the 36th week of gestation. Sudden and persistent vomiting is followed by abdominal pains, jaundice and tachycardia. There is no fever. The patient rapidly becomes somnolent and vomiting assumes a coffee ground aspect. Premature labor sets in, the patient delivers a stillborn child, lapses into coma and dies with a terminal temperature elevation usually 1 to 7 days post partum. Few patients have survived. Two survivals occurred after early Caesarian section. Serum bilirubin rarely surpasses 10 mg per 100 ml, alkaline phosphatase and transaminases are moderately elevated, prothrombin time is markedly prolonged, white cell counts vary between 20 000 and 30 000, and flocculation and turbidity reactions are normal. Hypoglycemic episodes may occur and oliguria with azotemia is not uncommon. A few patients had associated pancreatitis.

Both intrahepatic cholestasis of pregnancy and acute fatty metamorphosis of pregnancy do not occur outside of gestation and in both the etiology is unknown.

Hyperemesis gravidarum is occasionally associated with mild jaundice and rarely with pruritus. Pathological bile components may be present in the urine, the transaminases may be slightly elevated and occasionally the flocculation tests are positive. Histological liver changes are absent or nonspecific. Jaundice in hyperemesis has no prognostic significance and clears with the cessation of vomiting.

Toxemia of pregnancy is associated with a high incidence of abnormal liver function tests. Flocculation tests are positive in about half the cases. Alkaline phosphatase is elevated and parallels the clinical course. Transaminases are increased in the more severe cases. Jaundice is rare and carries a grave prognostic outlook. Histological liver changes are impressive at postmortem examination but absent or minor in liver biopsies. These changes appear to be a terminal event, reflect the basic vascular disorder and cannot be implicated in the genesis of the disturbed liver function tests.

The only disease in which an induced termination of pregnancy appears indicated because of jaundice is acute fatty metamorphosis of pregnancy. In order to save the patient it should be performed very early after the onset of symptoms. An interruption of pregnancy may become necessary in toxemia but indications are based on general obstetric principles. Interruption of pregnancy may also be indicated in some cases of hemolysis not responding to medical treatment, but the decision is based on the degree of anemia and not on the presence of jaundice. Interrup-

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PART III contains a review on recurrent jaundice during pregnancy A total of 132 cases are reported in the world literature For the purpose of this review the cases have been divided into 6 categories according to their documentation (see Table 10) In 43 cases the diagnosis of intrahepatic cholestasis of pregnancy has been accepted (categories I and II) In 28 cases a different disease entity is responsible for recurrent jaundice (category VI) In 61 cases documentation is insufficient for a critical evaluation (categories III—V) In addition, 267 cases of non-recurrent intrahepatic cholestasis of pregnancy are briefly mentioned (category VII)

The terminology formerly used for recurrent intrahepatic cholestasis of pregnancy includes icterus gravidarum, jaundice of pregnancy, idiopathic jaundice of pregnancy, recurrent jaundice of pregnancy, benign jaundice of pregnancy, idiopathic hepatopathy of pregnancy obstetric hepatosis endogenous hepatotoxemia of pregnancy and cholestatic jaundice of pregnancy

In 14 reports covering 23 patients the diagnosis of intrahepatic cholestasis of pregnancy is based on liver biopsies and can be accepted beyond reasonable doubt (category I) Adequate laboratory examinations and details on clinical course are reported in 18 of these cases (category I A) These patients underwent a total of 70 gestations, in 47 of which the full syndrome with pruritus and jaundice occurred The 47 pregnancies form the basis for establishing criteria of diagnosis in this disease

Liver biopsies reveal only a mild centroacinar cholestasis, which is focal or irregular Some bile canaliculi contain bile plugs They are of normal caliber or slightly dilated The surrounding liver cells contain biliary pigment The liver cells are intact, as are the portal fields There is no inflammation Minor changes are consistent with those seen in uncomplicated pregnancies Macroscopical findings in 10 patients undergoing surgical laparatomies were considered normal Only one patient had gall bladder stones In none was extra hepatic bile flow impaired Radiological gallbladder examinations show no filling during jaundice and are normal after its subsidence

Pruritus precedes jaundice usually by 1 to 2 weeks, in rare instances by up to 22 weeks Itching is violent involves the trunk and/or the extremities and leads to insomnia Mean onset of jaundice is in the 26th week with an observed range between the 7th and the 39th week Median duration of jaundice from its onset to spontaneous delivery is 6 weeks, and mean duration 8 1 weeks with an observed range from 1 to 33 weeks There are no prodromal symptoms such as in viral hepatitis Apart from itching there are no general symp

toms General well being is not impaired Physical examination is normal except for the presence of jaundice and scratch marks After delivery, whether *spontaneous* or induced jaundice disappears within 1 to 2 weeks in the majority of patients, but may last in some up to 4 weeks postpartum Itching subsides before jaundice Recovery is complete after delivery

Peak serum bilirubin is below 8 4 mg per 100 ml in all and below 6 mg per 100 ml in most cases The direct reacting bilirubin constitutes the major fraction Urobilinogen is never absent from the urine Urobilin and bilirubin are usually present Bilirubinuria may be transient and can be missed Alkaline phosphatase is increased to peak levels of 28 Bodansky units and serum cholesterol to maximally 590 mg per 100 ml One or both these parameters may be *normal in an occasional case* Serum electrophoresis shows an increase in alpha 2 and beta globulins and a decrease in serum albumin Gamma globulins are always lower than beta globulins Flocculation and turbidity test are normal Prothrombin time may be prolonged when jaundice is of long duration This is due to a deficiency in the Vitamin K dependent coagulation factors II VII and X Serum transaminases are elevated up to 250 units and reached 900 units in a single instance Bromsulfalein retention at 45 minutes is between 10 and 25 % Galactose tolerance is normal There is no evidence of hemolysis

After delivery there is a rapid decline of serum bilirubin and serum transaminases while alkaline phosphatase may continue to rise for 4 to 10 days post partum. There are no obstetrical complications In cases with prolonged *prothrombin time* blood loss during delivery may be excessive. The overall incidence of premature deliveries is high Interestingly enough, premature delivery is confined to about one third of the patients while the others deliver at term Premature delivery appears to be independent of jaundice per se It may be caused by the same metabolic disturbance which also causes intrahepatic cholestasis. Child survival depends on the degree of maturity No baby was icteric

In successive pregnancies the syndrome may increase or decrease in severity or may present repeatedly with the same intensity Clinical course regarding onset of jaundice and date of delivery is similar in many patients

Treatment consists in the prophylactic application of Vitamin K and in the administration of cholestyramine to relieve itching Neither diet nor bed rest are necessary and the patient may continue her usual daily life

In some instances there are 2 or more cases of recurrent intrahepatic jaundice *of pregnancy in close relatives* No antecedent liver disease is responsible for its occurrence

Pruritus gravidarum appears to be a forme fruste of the full syndrome and may show similar changes in alkaline phosphatase serum cholesterol and serum transaminases

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The differential diagnosis of recurrent jaundice during pregnancy is given in Table 23 Recurrent jaundice during pregnancy may probably occur in all benign disorders causing jaundice during pregnancy Documented examples have been published for hemolytic jaundice, familial non hemolytic jaundice, post hepatitic hyperbilirubinemia, gall stone obstruction of the common bile duct, jaundice in severe pyelonephritis and *jaundice in hyperemesis gravidarum* Cases with serum bilirubin levels above 10 mg per 100 ml represent mostly exacerbations of chronic anicteric hepatitis under the stress of pregnancy Recurrent jaundice during pregnancy may be due to different diseases in successive gestations, for instance hepatitis in the first and hemolytic jaundice in the second gestation, or the etiology may be mixed in a single gestation, for instance hepatitis combined with hemolysis Six recorded cases in the literature could not be classified at all

The diagnosis of intrahepatic cholestasis of pregnancy should only be made when the criteria outlined above are met and when the differential diagnosis of recurrent jaundice during pregnancy has been carefully considered

REFERENCES

*** cases of recurrent intrahepatic cholestasis of pregnancy with liver biopsy (category I)

** cases of recurrent intrahepatic cholestasis of pregnancy without liver biopsy but adequate laboratory data (category II)

* cases of recurrent jaundice during pregnancy with inadequate documentation (categories III—V)

° cases of non recurrent intrahepatic cholestasis of pregnancy (category VII)

+ cases with recurrent jaundice during pregnancy of an etiology other than recurrent intrahepatic cholestasis of pregnancy (category VI)

Abrams F R Cirrhosis of the liver in pregnancy A review of the literature and report of a case with electrophoretic studies. Obstet. Gynec 10 451—456 1956

Adno J Liver cirrhosis and pregnancy Report of a case following spleno-renal shunt South African M J 31 1189- 1191 1957

* Ahlfeld F Icterus gravidarum Berichte u Arbeiten aus der Geb Gyn. Klinik zu Giessen 1881—1882 pp 148—153

* Ahlfeld F Lehrbuch der Geburtshilfe W Grunow Leipzig 1898 2 Aufl p 242

Ahrens E H Payne M H Kunkel, H G Eisenmenger W J and Blondheim S H Primary biliary cirrhosis Medicine 29 299—364 1950

+ Albano V and Albano S B L epatite virale in gravidanza Sicilia Sanit 4 95—116 1962

Alex R Ikterus in der Schwangerschaft (Ein kasuistischer Beitrag) Zbl Gynak 73 1324—1351 1951

Antia, F P Bharadwaj T P Watsa M C and Master J Liver in normal pregnancy pre-eclampsia and eclampsia Lancet ? 776—778 1958

Arfwedson H Graviditetsklåda Svenska Lak Tidningen 50 1685—1689 1953

Arfwedson H General pruritus in pregnancy Symptom of liver dysfunction Obstet Gynec 7 274—? 6 1956

Arfwedson H and v Studnitz W Ueber bei Schwangerschaftspruritus auftretende Veranderungen des Lipoprotein Lipoid und Proteingehaltes im Serum Klin Wschr 34 183—185 1956

Ashton D L Banti s disease complicating the puerperium Am J Obstet Gynec. 28 280—281 1934

Baens A and Espinola N Acute yellow atrophy of the liver in pregnancy report of cases. J Philippine Med. Ass. 17 679—685 1937

° Barry A. P and O Dwyer, E. Hepatitis in pregnancy Irish J Med Sci 6 419—424 1955

Bean W B The cutaneous arterial spider Survey Medicine 24 243—331 1945

Bean W B Cogswell, R Dexter M and Embick, J 1 Vascular changes of the skin in pregnancy Vascular spiders and palmar erythema. Surg Gynec. Obstet. 88 739—752 1949

Bearn A. G Kunkel, H C and Slater, R J Problem of chronic liver disease in young women Amer J Med 21 3—15 1956

Beck, E. and Clark, L. C. Plasma alkaline phosphatase II Normal data for pregnancy Amer J Obstet Gynec 60 731—740 1950

* Becking A. G T Icterus gravidarum Bijdrage tot de pathologie der zwangerschap Tijdschr voor Verlosk en Gynaec 7 275—284 1896

*** Belvederi C and Fiorotti A. Sulla patogenesi dell ittero ricorrente gravidico. Riv Ital Ginec. 45 108—123 1961

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REFERENCES

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Abrams F R. C rhosis of the l er in p gna cy A re ew of the li era ure and report of a case w th elec ropho etic s udies Obstet. Gynec *10* 451—456 19 6

Ad o J Li er cirrhosis and pregnancy Report of a case follow ng spleno-renal shun South African M J *31* 1189—1191 1957

* Ahlfeld F Ic erus gra darum Berich e u Arbe en aus der Geb Gyn Klin k zu G essen 1881—1882 pp 148—153

* Ahlfeld F Lehrbuch der Geburtshilfe W Grunow Le pz g 1898 2 Aufl p 242

Ahrens E. H Payne M H Kunkel, H G Eise. me. ger W J and Bla. d eim S H Primary b ary irrhosis Med cine *29* 299—364 1950

+ Alba. o V and Alba. o S B L epa e irale n gra danza S c lia Sani *4* 95—116 1962

Allx R Ik erus n der Schwangerschaf Ein kasuis ischer Be trag Ztl Gynak *73* 13 4—1351 1951

A tia. F P B arad aj T P Watsa, M C and Master J Li e n normal p gnancy p e-eclampsia and eclampsia Lan e 7 6— 8 19 8

Arfwedson H Gra d etsklåda. S enska Läk T dn ngen *50* 1685—1689 1953

Arfwedson H General p u us in p egnancy S mp m of li er d sfunc on Obs e G nec 7 274—2 6 1956

Arfwedso H and Stud tz W Ueber be Schwangerschaftspruri us auf re ende Veranderungen des Lipopro ein Lipo d und Proteingehaltes im Serum Klin Wschr *34* 183—185 1956

Ashton D L. Banti s disease complica ng he puerperium Am J Obs et. Gynec *78* 280—281 1934

Baens A and Espinola N Acu e yellow atrophy of he li er in pregnancy report of cases. J Ph l ppine Med Ass. *17* 6 9—683 1937

° Barry A. P and O Dwyer, E. Hepa tis n p egnancy Irish J Med. Sci *6* 419—424 1953

Bean W B The cu aneous arterial sp der A survey Medicine *24* 243—331 1945

Bean W B Cogswell, R Dexter, M and Emb ck J F Vascular changes of the skin n pregnancy Vascular sp ders and palmar erythema Surg Gynec Obs et. *88* 739—752 1949

Bearn A G Kunkel, H G and Slater, R J Problem of chron c l er disease in young women Amer J Med *21* 3—15 1956

Beck, E. and Clark L. C. Plasma alkaline phosphatase II Normal data for p egnancy Amer J Obs et Gynec *60* 731—740 19 0

* Becking A G T Icterus gra darum. B jd age ot de pathologie der zwangerschap T jdschr oor Verlosk. en Gynaec 7 275 284 1896

** Bel ederi C and Finotti A. Sulla patogenes dell ero ricorren e gra d co Ri Ital Ginec *45* 108—123 1961

lesterol, electrophoresis) is marked, but structural cholestasis (liver biopsy) is minimal "Physiological" derangement of liver function in normal pregnancy, pruritus gravidarum and intrahepatic cholestasis of pregnancy appear to be but increasing manifestations of the same basic disorder, and intrahepatic cholestasis of pregnancy is considered to be but an exaggeration of a 'normal" process during gestation

The differential diagnosis of recurrent jaundice during pregnancy is given in Table 23 Recurrent jaundice during pregnancy may probably occur in all benign disorders causing jaundice during pregnancy Documented examples have been published for hemolytic jaundice, familial non hemolytic jaundice, post hepatitic hyperbilirubinemia, gall stone obstruction of the common bile duct, jaundice in severe pyelonephritis and jaundice in hyperemesis gravidarum Cases with serum bilirubin levels above 10 mg per 100 ml represent mostly exacerbations of chronic anicteric hepatitis under the stress of pregnancy Recurrent jaundice during pregnancy may be due to different diseases in successive gestations, for instance hepatitis in the first and hemolytic jaundice in the second gestation, or the etiology may be mixed in a single gestation, for instance hepatitis combined with hemolysis Six recorded cases in the literature could not be classified at all

The diagnosis of intrahepatic cholestasis of pregnancy should only be made when the criteria outlined above are met and when the differential diagnosis of recurrent jaundice during pregnancy has been carefully considered

and the bromsulfalein tests Amer J Obstet. Gynec. 59 1100—1104 1950

Clinicopathologic conference. *Fulminating* liver disease in a pregnant woman at term Amer J Med 35 231—240 1963

COMBES B SHIBATA, H. ADAMS R MITCHELL, B D and TRAMMELL V Alterations in sulfobromophthalein sodium removal *mechanisms from* blood during normal pregnancy J Clin Invest 42 1431—1442 1963

° COMERFORD J B The diagnosis and treatment of hepatitis in pregnancy J Obstet. Gynaec Brit. Cwlth 69 1022—1028 1962

COROOS A. Ictère grave et grossesse (A propos de 8 cas) Presse med 62 544—(544) 1954

CORYELL, M N BEACH E F ROBINSON A R MACY I G and MACK H C Metabolism of women during the reproductive cycle XVII Changes in electrophoretic patterns of plasma proteins throughout the cycle and following delivery J Clin Invest 29 1559—1567 1950

CREMONA G F and VOCHERA C Considerazioni cliniche sulle epatopatie itterigene in gravidanza Minerva Ginec 14 1199 —1212 1962

CRISMER R Ictère hémolytique acquis toxigravidique Acta gastro-enterol Belg 15 549—553 1952

CRISP W E MIESFELD R L and FRAJOLA, W J Serum glutamic oxalacetic transaminase levels in the toxemias of pregnancy Obstet Gynec 13 487—497 1959

CROSS R C A study of various liver function tests in normal pregnancy Amer J Obstet Gynec 18 800—807 1929

CULLINAN E R Idiopathic jaundice (often recurrent associated with subacute necrosis of the liver St Bart Hosp Rep 69 55—142 1936

CURTIS E M Pregnancy in sickle-cell anemia sickle-cell hemoglobin C disease and variants thereof Amer J Obstet Gynec 77 1312—1323 1959

DAMESHEK W and SCHWARTZ S O Acute hemolytic anemia (acquired hemolytic icterus, acute type) Medicine 19 231—327 1940

DAY E M A and HELLESTRAND A L An investigation of liver function in normal pregnancy and in the late toxemias of pregnancy Australian Med. J 2, 326—329, 1947

DEGAUDIN E Concommitance des maladies du foie et des reins et en particulier des reins dans l ictere V A Delahaye et Cie Paris 1878 pp 117

DE GROOTE J GOUBEAU P and VANDENBROUCKE J Ictere cholostatique recidivant Acta gastroenterol Belg 23 747—755, 1960

DENNING H and BRUCKMANN R. Virushepatitis und Schwangerschaft Munch med Wschr 103 1817—1821 1961

DIECKMANN W J The toxemias of pregnancy C V Mosby Company St Louis 2nd ed 1952

DIECKMANN W J SMITTER R. C and POTTINGER, R. E. Liver function studies in normal and toxemic pregnancy Surg Gynec Obstet 92 598—600 1951

DIECKMANN W J and POTTINGER R E. Serial studies of the cephalin flocculation and thymol turbidity tests in pregnant patients. Amer J Obstet Gynec. 68 1581—1583 1954

DIETEL, H Die Leberfunktionsprufung in der Schwangerschaft Ztschr Geburtsh Gynak 113 209—254 1936

DIETEL, H Das Bild der Leber in der Schwangerschaft (dargestellt auf Grund bioptischer Untersuchungen) Ztschr Geburtsh Gynak. 128 127 162 1947

DIETEL, H Hepatitis epidemica und Schwangerschaft Geburtsh Frauenheilk 12 525—534 1952

DIETEL, H Storungen und Erkrankungen der weiblichen Geschlechtsorgane in ihren Beziehungen zur Leber und Gallenblase in Seitz L and Amreich A I Biologie und Pathologie des Weibes. Ein Handbuch der Frauenheilkunde und Geburtshilfe 2 Aufl Bd. 6 Teil 3 Verlag Urban & Schwarzenberg Berlin Munchen Wien 1954

• • DIETEL, H Der idiopathische Schwangerschaftsikterus. Geburtsh Frauenheilk 22 + 505—510 1962

BENARON, H B W, DORR, E M, RODDICK, W J, JOHNSON R P, GOSSACK, L and TUCKER, B E The use of chlorpromazine in the obstetric patient a preliminary report I In the treatment of nausea and vomiting of pregnancy Amer J Obstet Gynec *69*, 776—779, 1955

+ BENEDICT, H Zur Kenntnis des Schwangerschaftsikterus Dtsch med Wschr *28*, 296—297 1902

BENNET, A G, KUNIN A S and WALLACE, H J Liver disease in pregnancy report of a case complicated by jaundice and cirrhosis with a review of the literature Obstet Gynec Survey *18* 919—934, 1963

*** BÉRAUD C, BROUQUET MARTIN, C, PACCALIN and TRAISSAC F J L ictere recidivant de la grossesse *Arch Mal* Appar Dig *52*, 256—261, 1963

BJERRIGAARD P C Tilfaelde of Svangerskabs Ikterus Ugeskrift for Lager 5 R XI, 895—898 1904

BODANSKY, M CAMPBELL, K and BALL, E Changes in serum calcium inorganic phosphorus and phosphatase activity in the pregnant woman Amer J Clin Path *9* 36—51 1939

+ BOQUIEN Y CUILLON J LERAT M and LENNE C Les icteres de la grossesse Gynec Obstét (Paris) *60* 562—584 1961

* BOREEL M Over een geval van icterus gravidarum Ned Tijdschr Geneesk *68* 1018—1022 1924

BORGLIN N E Serum transaminase activity in uncomplicated and complicated pregnancy and in newborns J Clin Endocrin *18* 872—877 1958

+ BRAUER L Ueber Graviditats Haemoglobinurie Munch med Wschr *49* 825—826 *1902*

* BRAUER L Ueber Graviditatsikterus Zbl Gynak *27*, 787—789 1903

BRET J and SENEZE J Les icteres au cours de la grossesse Rev int Hépat *7* 557—578, 1957

BRET J and SÉNEZE, J Les icteres au cours de la grossesse Rev pract (Paris) *8* 963—973, 1958

BROCQ PORTES and VARANGOT Ictere chronique par retention d origine lithiasique au cours de la grossesse cure chirurgicale guerison Gynec Obstet (Paris) *42* 120—121, 1942

+ BROMBERG, Y M TOAFF R and EHPENFELD, E Acute crises in chronic hemolytic anemia induced by pregnancy J Obstet Gynaec Brit Emp *55* 325—329 1948

° BROWN, D F PORTA E A and REDER, J Idiopathic jaundice of pregnancy Clinical chemical and ultrastructural hepatic changes in three cases Arch Int Med *111* 592—606 1963

BRUNO M S and OBER, W B Jaundice at the end of pregnancy Clinico-pathological conference New York State J Med *62* 3792—3800 1962

BUDD, G bearbeitet von Henoch L. H Die Krankheiten der Leber A Hirschwald Berlin, 1846 p 432

BURSLEM R W GARDIKAS C and ISRAELS M C G Liver cirrhosis and pregnancy J Obstet Gynaec Brit Emp *59* 777—782 1952

*** CAHILL, K M Hepatitis in pregnancy
* Surg Gynec. Obstet *114* 545—552 1962

CANTAROW, A, STUCKERT H and GARTMAN E Studies of hepatic function IV Hepatic function during pregnancy Amer J Obstet Gynec *29* 36—43 1935

CAROLI J PARAF A and ZERVOYANNIS S Forme cholostatique pure des hepatites icterogenes Rev med Suisse rom *73* 929—963 1953

* CAROLI J PUJO G and RAMPON Remarques sur les hepatites icterigenes de la
+ grossesse Semaine Hop Paris *30* 1692—1699 1954

CATTAN R and CATTAN A Ictere et grossesse Rev int Hepat *9* 399—416 1959

CAYLA J and FABRE F La phosphatase serique pendant la gestation Compte rend Soc Biol *120* 748—750 1935

+ CHABROL E Les icteres Masson & Cie Paris 1932 p 266

CHRISTHILF S M and BONSNES R W Liver function during pregnancy and the puerperium as measured by the cephalin cholesterol flocculation the thymol turbidity

and the bromsulfalein tests Amer J Obstet. Gynec 59 1100—1104 1950

Clinicopathologic conference Fulminating liver disease in a pregnant woman at term Amer J Med. 35 231—240 1963

COMBES B SHIBATA, H. ADAMS R. MITCHELL, B D and TRAMMELL V Alterations in sulfobromophthalein sodium removal mechanisms from blood during normal pregnancy J Clin Invest 42 1431—1442 1963

° COMERFORD J B The diagnosis and treatment of hepatitis in pregnancy J Obstet Gynaec. Brit Cwlth 69 1022—1028 1962

CORCOS A Ictère grave et grossesse (A propos de 8 cas) Presse med 62 544—(544) 1954

CORYELL M N BEACH E F ROBINSON A R MACY I G and MACK H C Metabolism of women during the reproductive cycle XVII Changes in electrophoretic patterns of plasma proteins throughout the cycle and following delivery J Clin Invest 29 1559—1567 1950

CREMONA, G F and VOGHERA G Considerazioni cliniche sulle epatopatie itterigene in gravidanza Minerva Ginec 14 1199—1212 1962

CRISMER R Ictere hémolytique acquis toxi gravid que Acta gastro-enterol Belg 15 549—553 1952

CRISP W E MIESFELD R I and FRAJOLA W J Serum glutamic oxalacetic transaminase levels in the toxemias of pregnancy Obstet Gynec 13 487—497 1959

CROSS R C A study of various liver function tests in normal pregnancy Amer J Obstet Gynec 18 800—807 1929

CULLINAN E R Idiopathic jaundice (often recurrent) associated with subacute necrosis of the liver St Bart Hosp Rep 69 55—147 1936

CURTIS E M Pregnancy in sickle-cell anemia sickle-cell hemoglobin C disease and variants thereof Amer J Obstet Gynec 77 1312—1323 1959

DAMESHEK, W and SCHWARTZ S O Acute hemolytic anemia (acquired hemolytic icterus, acute type) Medicine 19 231—327 1940

DAY E. M A and HELLESTRAND A. L. An investigation of liver function in normal pregnancy and in the late toxemias of pregnancy Australian Med J 2 326—329, 1947

DECAUDIN E Concommitance des maladies du foie et des reins et en particulier des reins dans l ictere V A Delahaye et Cie Paris 1878 pp 117

DE GROOTE J GOUBEAU P and VANDENBROUCKE J Ictere cholostatique recidivant Acta gastroenterol Belg 23 747—755 1960

DENNING H and BRUCKMANN R Virushepatitis und Schwangerschaft Munch med Wschr 103 1817—1821 1961

DIECKMANN W J The toxemias of pregnancy C. V Mosby Company St Louis 2nd ed 1952

DIECKMANN W J SMITTER, R C and POTTINGER R E Liver function studies in normal and toxemic pregnancy Surg Gynec Obstet 92 598—600 1951

DIECKMANN W J and POTTINGER, R E Serial studies of the cephalin flocculation and thymol turbidity tests in pregnant patients. Amer J Obstet Gynec 68 1581—1583 1954

DIETEL, H Die Leberfunktionsprufung in der Schwangerschaft Ztschr Geburtsh Gynak 113 209—254 1936

DIETEL, H Das Bild der Leber in der Schwangerschaft (dargestellt auf Grund bioptischer Untersuchungen) Ztschr Geburtsh Gynak. 128 127—162 1947

DIETEL, H Hepatitis epidemica und Schwangerschaft Geburtsh Frauenheilk 12 525—534 1952

DIETEL, H Storungen und Erkrankungen der weiblichen Geschlechtsorgane in ihren Beziehungen zur Leber und Gallenblase in Seitz L. and Amreich, A.I Biologie und Pathologie des Weibes. Ein Handbuch der Frauenheilkunde und Geburtshilfe 2 Aufl Bd 6 Teil 3 Verlag Urban & Schwarzenberg Berlin Munchen Wien 1954

••• DIETEL, H Der idiopathische Schwanger
\+ schaftsikterus. Geburtsh Frauenheilk. 22 505—510 1962

DILL, L V Acute yellow atrophy of the liver associated with pregnancy, a review of the literature and six cases *Obstet Gynec Surv 5, 139—158* 1950

*** DÖLLE, W and MARTINI G A Gelbsucht mit Verschlussyndrom als Leitsymptom bei Virushepatitis, Arzneimittelschaden, in der Schwangerschaft und bei Neugeborenen Acta hepato-splen *6*, 138—155, 255—247, 1959

DOLLE W and MARTINI, G A Zusammenstellung von Arzneimitteln, die Leberschädigung mit und ohne Gelbsucht verursachen konnen (Erganzung) Acta hepato-splen *9* 74—85 1962

DOMINICI, G Le malattie del fegato e delle vie biliari F Vallardi, Milano 3 ed Vol I 1960, p 720—721

DORFLER, R Zur Frage der kindlichen Missbildungen infolge Erkrankung der Mutter an Hepatitis epidemica wahrend der Schwangerschaft *Munch med Wschr 99,* 1664—1667, 1957

DORFLER R Ueber die Haufigkeit hepatischer Embryopathien Aerztl *Wschr 13,* 779—781 1958

* DOWIE R *A case of jaundice recurring in* pregnancy *J Obstet* Gynaec *Brit* Emp *61*, 399—(399) 1954

DUBIN, I N Chronic idiopathic jaundice A review of fifty cases Amer J Med *24* *268—292* 1958

DUBIN, I N SULLIVAN, B H LE GOLVAN P C and MURPHY L C The cholostatic form of viral hepatitis Experiences with viral hepatitis at Brooke Army Hospital *during the years 1951 to 1953 Amer J Med* 29 55—72 1960

DUNCAN C and MACLACHLAN G R Report of a case of yellow atrophy of the liver in the latter part of pregnancy with recovery *Amer J Obstet Gynec 25* 157—158 1933

DURST M and STRAUSS B Transaminasenbestimmungen in der Geburtshilfe Geburtsh Frauenheilk 23 927—934 1963

DYSON B C Fatty metamorphosis of the liver in pregnancy report of a fatal case without jaundice Bull Ayer Clin Lab Penn Hosp *4* 17—30 1959

EDWARDS, A G A case of obstetrical acute yellow atrophy of the liver J Obstet Gynaec Brit *Emp 67, 460—462, 1960*

ELLEGAST H, GUMPESBERGER, G and WEWALKA F *Hepatitis in der Graviditat* und Fruhgeburt. *Wien Klin Wschr 66* *30—32*, 1954a

ELLEGAST, H, GUMPESBERGER, G RISSEL, E. and WEWALKA, F Virushepatitis und Graviditat Haufigkeit und Schwere der Hepatitis Wien Klin Wschr *66 42—48 1954b*

ELLEGAST, H GUMPESBERGER G and WEWALKA F Einfluss einer Hepatitis in der Schwangerschaft auf das Kind Wien Klin *Wschr 66 507—511, 1954 c*

ENRILE, R R., ARELLANO S O TAMAYO, J G and SANTOS D C *Jaundice in pregnancy* J Philippine Med Ass *33* *587—602, 1957*

* EPPINGER H Icterus gravidarum in Kraus P and Brugsch T *Spezielle Pathologie und Therapie innerer Krankheiten Bd* 6, 2 Halfte III Teil Urban & Schwarzenberg Berlin/Wien, 1923, pp 305—306

EPPINGER H Die Leberkrankheiten Allgemeine und spezielle Pathologie und *Therapie der Leber Julius Springer* Wien, 1937 pp 532—535

ERNAELSTEEN D and WILLIAMS R Jaundice due to Nitrofurantoin Gastroenterology *41* *590—593 1961*

EUFINGER H and BADER C W *Pigment*stoffwechsel der Leber in der Schwangerschaft Arch Gynak *128 293—308 1926*

EUFINGER H and BADER C W Die Bedeutung der H v d Berg schen Probe in der *Schwangerschaft* insbesondere bei Toxikosen Zbl Gynak *50 514—517 1926*

+ EZES H and BOURDON R Les hepatites icterigenes graves d allure virale au cours de la gestation (Synthese critique de 36 observations recueillies de 1952—1956) Gynec Obstet (Paris) *55 288—311 1956*

FERRU M L ictere dans *les vomissements* graves de la grossesse *These* Paris 1926

FOUCHÉ H H and SWITZER P K *Preg*nancy with sickle cell anemia Review of the literature and report of cases Amer J Obstet Gynec *58 468—477* 1949

Foulk W T Butt H R Owen C A Whitcomb F F and Mason H L Constitutional hepatic dysfunction (Gilbert's Disease) its natural history and related syndromes Medicine 38 25—46 1959

Frerichs F T Klinik der Leberkrankheiten Verlag F Vieweg Braunschweig 1858 Bd I pp 700—201

Friedberg V Ueber die latenten Schwangerschaftshepatosen Z. Geburtsh Gynak *134* 27—39 1951

Friedberg V Die Leberfunktion in der Schwangerschaft Acta hepato-splen 7 214—229 1960

* Friedberg V Ueber die Bedeutung der Leberfunktionsprufungen in der Schwangerschaft Geburtsh Frauenheilk *22* 109—122 1962

Friedberg V Die Leberfunktion in der Schwangerschaft Munch med Wschr *105* 58—64 1963

Friedman M M Lapan B and Taylor T H Variations of enzyme activities during normal pregnancy Amer J Obstet. Gynec *82* 132—137 1961

Frucht H L and Metcalfe, J Mortality and late results of infectious hepatitis in pregnant women New Engl J Med *25* *1094—1096 1954*

* Fruhinsholz A A propos de la pyélite gravidique un cas de syndrome pyélo-hepato-renal récidivant à deux gestations successives. Bull Soc Obstét Gynéc *18* *141—145 1929*

* Cabriel H and Bernardin D Treize observations d'ictère au cours de la grossesse Bull Fed Gynec Obstét Franç *14* 395—398 1962

Gall, F A and Braunstein H Hepatitis with manifestations simulating bile duct obstruction (so-called cholangiolitic hepatitis) Amer J Clin Path *25* 1113—1127 1955

Gebhardt W F Van Ommen R A McCormack, L J and Brown C H Chlorpromazine jaundice Clinical course hepatic function tests, and pathological findings — summary of twenty cases. Arch Int Med *101* 1085—1093 1958

Gerl, D and Bonow, A Zur Klinik und Differentialdiagnose des Ikterus der Schwangeren Med. Klin *59* 1—6 1954

Goldstein, K. and Contino C A Intrahepatic cholestasis of pregnancy Bull Millard Fillmore Hosp 9 75—78 1962

*** Gros, H Der recidivierende idiopathische Schwangerschaftsikterus Dtsch med Wschr *83* 383—386, 1958

Gros H Personal communication 1964

Guttmacher, A F Hyperemesis. in Guttmacher A F and Rovinsky J J pp 166-169

Guttmacher, A. F and Rovinsky J J Medical surgical and gynecological complications of pregnancy Williams & Wilkins Company Baltimore 1960

*** Haemmerli U P and Wyss, H I Re
** current intrahepatic cholestasis of pregnancy Report of six cases, with liver biopsies in four (Manuscript submitted for publication)

° Haemmerli U P and Wyss H I unpublished observations.

von Harnack, G A. and Martini, G A. Hepatitis und Schwangerschaft. Die Auswirkung der Hepatitis auf die Frucht. Dtsch med Wschr *77* 40—42 1952

Hartmann F and Schoen R. Hepatitis in der Schwangerschaft Geburtsh Frauenheilk. *15* 305—312 1955

*** Hausheer H J and Lauer D J Recurrent jaundice of pregnancy Report of a case New Engl J Med *267* 1300—1301 1962

Haverback, B J and Wirtschafter, S K Familial nonhemolytic jaundice with normal liver histology and conjugated bilirubin. New Engl. J Med. *262* 113—117 1960

Herold L. Die Leberfunktion in der physiologischen Schwangerschaft und bei der Hyperemesis gravidarum Arch Gynak, *168* 509—524 1939

Hesseltine, H C. Splenomegaly with hepatic cirrhosis (Banti's syndrome) as a complication of pregnancy with the report of a case. Amer J Obstet. Gynec. 20 77—80 1930

Hill, J H Serum lactic dehydrogenase in cancer patients J Nat Cancer Institute *18* 307—313 *1957*

Hoch H. Marrack, J R. Rose, R. H *and Hoch R.* The composition of the blood

of women during pregnancy and after deli very J Obstet Gynaec Brit Emp 55, 1—16 1948

HOFBAUER, J Beitrage zur Aetiologie und zur Klinik der Graviditatstoxicosen (Cholamie, Eklampsie, Hyperemese) Z Geburtsh Gynak 61, 200—274, 1907

* HOFFMAN cited in MEUNIER J Essay critique sur l ictère des femmes enceintes à propos de l epidemie de Paris 1871—1872 These Paris 1872, p 17

* HOLMER, A J M Lever en zwangerschap Ned Tijdschr Verlosk en Gynaec 32, 296—308 1927

HORN G Observations on the aetiology of cholelithiasis Brit Med J 2 732—737 1956

HOLEL P, FABRECOLLE, M GARES R and BOURDON R Les formes malignes de l hepatite virale au cours de la gestation Sem Hop Paris 34 1037—1043 1958

HOYNCK VAN PAPENDRECHT, H P C M Leverfunctiestoornissen in het laatste trimester van de zwangerschap Ned Tijdschr Verlosk en Gynec 57 403—410 1957

HSIA D Y TAYLOR, R G and GELLIS S S A long term follow up study on infectious hepatitis during pregnancy J Pediat 41 13—17 1952

*** IKONEN E Jaundice in late pregancy Acta
* obstet gynec Scand 43 Suppl 5 1—130
° 1964
+

IMPARATO E Gli itteri in gravidanza Rass int Clin Therap 38 557—558 1958

INGERSLEV M and TEILUM G Biopsy studies on the liver in pregnancy I Normal histological features of the liver as seen on aspiration biopsy Acta obstet gynec Scand 25 339—351 1945

INGERSLEV M and TEILUM G Biopsy studies on the liver in pregnancy II Liver biopsy on normal pregnant women Acta obstet gynec Scand 25 352—360 1945

INGERSLEV M and TEILUM G Biopsy studies on the liver in pregnancy III Liver biopsy in albuminuria of pregnancy eclampsism and eclampsia Acta obstet gynec Scand 25 361—376 1945

INGERSLEV, M and TEILUM G Jaundice during pregnancy Acta obstet. gynec. Scand 31, 74—89, 1951

JAVERT, C. T and MORRISON R C Jaundice II Its relationsship to pregnancy Texas J Med 47 137—141 1951

JEFFRIES, G H and SLEISENGER, M H Acute intrahepatic cholestasis Med Clin N America 44, 623—632 1960

** JODKOWSKI H and CHOJECKA, B Nawracajaca zoltaczka w przebiegu ciazy Pol Tyk Lek 16, 891—893, 1961

JOHN C G and KNUDTSON K P Chronic idiopathic jaundice, two cases occurring in siblings with histochemical studies Amer J Med 21 138—142 1956

+ JUSTIN BESANÇON L PEQUINOT H ETIENNE J P and PHILBERT M L ictere gravidique recidivant Rev med Franç 40 75—83 1959

KAHIL M E FRED H L BROWN H and DAVIS J S Acute fatty liver of pregnancy Report of two cases Arch Int Med 113 63—69 1964

KALK H Ueber die posthepatitische Hyperbilirubinamie Gastroenterologia 84 207—225 1955

KASDON S C Abdominal pruritus in pregnancy Amer J Obstet Gynec 65 320—324 1953

** KATZ R VELASCO M MANZUR F and
° DONOSO S Ictericia del embarazo Rev Med Chile 89 110—115 1961

KAUFMANN C Ueber die Schwangerschaftsleber Z Geburtsh Gynak 99 582—588 1931

KAUFMANN C Ueber die Schwangerschaftsumstellung der Leberfunktion Klin Wschr 11 493—495 1932

* KEHRER F Zur Lehre von der embryogenen Toxaemia gravidarum Klin Vortrage N F Nr 398 Serie 15 365—404 1905

KEHRER E Die Bedeutung des Ikterus in der Schwangerschaft fur Mutter und Kind Klinische und experimentell Untersuchungen Arch Gynaec 81 129—159 1907

KELLOG C S and WESP J E Infectious hepatitis during pregnancy and its effect upon the fetus Amer J Obstet Gynec 62 1153—1156 1951

*** King M J and Kerrins J F Recurren d opathic jaundice of p egnancy New Eng J Med *68* 1180—1182 1963

Klajman A and Efrati P Prolonged jaund ce wi h unidentified p gmen n li er cells Lancet *1* 538—539 1955

Klier, E Ik erus im Rahmen der Schwanger schaftsf uh ox cose W en klin Wschr *68* 64—66 1956

K utson R G Cornatzer, W E. Moore J H and Nelso W W Serum la uc dehyd ogenase and glutam c oxala etic transaminase a vi es n normal p egnancy J Lab Clin Med *51* 773—777 1958

Kra l, L. Lebercirrhose und S l wanger *schaf Zbl* Gynak *51 663—669* 1927

K bli F T ansamina e M lchsauredehy dr genase und a kalische Phosphatase n der Spatschwangerschaf un er de Gebur und be Toxikose Gynaecologia *151* 72 4 961

K H A Ik erus dur h ntrahepa cl e Cho es ase be B udern Acta hepa o- plen 9 229—245 1962

Kun S F and Breci er, G D ffe ential neu oph l counts n p egnancy New Eng J Med *266 877—878 1962*

Labby D H Li e fun on and primary er disease n p egnancy Gen Prac *22* 114 123 960

Labo G Facci M and Raiti F Le epa acu e gene n gra danza S ud o funziona e d l fega o g a d o Bologna Med 45— 68 19 9

Lacomme M I re c gestation Ma ern e 47 58 195

Lantue o l P and C iambra d R Accou hements p ma u és à épe on pa is fisance h pa que Gynec Obs e Paris *53* 305—308 9 4

Large A M Lo strom J E and S e e. son C S C a ls ones an l p egnancy Ar h Su g *8* 966 968 19 9

Lasc o J C. and Pereyra J C C *an* asc is por rrosis hepá a y embarazo Pr usa méd. Argen *23* 1794 800 1936

° La rijn ns M and Deme lenaere, L. I hepa hische erus b j de zwangerschap Be g T jdsch Geneesk *18* 946—9 1962

+ Lebon J Claude R *Trigo re J Galley* P and Leute. egger, M Ic ere gra d que *recidi an Algerie* Med *65* 235—244 1961

Lepage F Contribution a l e ude de la *pyélonephr e d e«gra do- oxique»* These Paris 1934

Le is P L. Takeda, M and Warren W J Obs etric acu e yellow atrophy Re port of a case Obs et. Gynec *2?* 121—127 1963

Ley H and Liebl, R Ueber Ausmass und Hauf gke on diffusen Hepa opath en nach einem Ik erus wahrend der Schwanger schaf Aerz Forschung *8* 451—457 1954

Lichtma S S Diseases of the l er gall bladder and b le ducts Lea and Feb ger Ph ladelphia 3 d ed 1953 pp 636—644

Li ton E B and Miller E. C Serum lact c dehyd ogenase in pregnancy Amer J Obs et Gynec *78* 11—12 1959

*** Ljunggren G Kliniska synpunk er på ° k erus under gra di et. Nord. med *55* 373—374 19 6

Lock F R B rt R L and Lide, T N Hepa c fa lure in p egnancy Amer J Obs et Gynec *65* 859—872 1953

Lo g J S Bossen *H* and Priest F O Infectious hepati is and p egnancy Amer J Obs e Gynec *0* 282 287 1955

Lo e W and Peel, E L Chlorp omazine aund ce in p egnancy J Obs et Gynaec Bri Cwlth *68* 623—633 1961

Lo rich J Zwei Fä le on Ik erus wahrend der Schwangerschaf Zbl Gynak *28* 7 6— 7 6 1904

Lowe stein L Brunton L Ya g-S t H and M lad A A. Fol c acid and V tamin B defic ency n *megaloblast c anemia of* p egnancy Amer J D g D s. (NS) *7* 984—985 1962

Mack, H C. Segar L F Robl so A. R Widema M E and Moger, E. Z El opho etic pa erns of plasma pro eins *n pregnancy II* Pregnancy comp ca ed by l er disease Obs et Gynec *1* 204—211 19 3

* McAlli ter, J E and Waddell, J M Recurring d opath c jaund ce n pregnancy case epo Amer J Obs e Gynec. *84* 62—64 1962

of women during pregnancy and after delivery J Obstet Gynaec Brit Emp 55 1—16, 1948

HOFBAUER, J Beitrage zur Aetiologie und zur Klinik der Graviditatstoxicosen (Cholamie, Eklampsie Hyperemese) Z Geburtsh Gynak 61, 200—274 1907

* HOFFMAN, cited in MEUNIER J Essay critique sur l ictere des femmes enceintes à propos de l epidemie de Paris 1871—1872 These, Paris 1872, p 17

* HOLMER A J M Lever en zwangerschap Ned Tijdschr Verlosk en Gynaec 32, 296—308, 1927

HORN, G Observations on the aetiology of cholelithiasis Brit Med J 2 732—737 1956

HOUEL, P, FABREGOULE, M, GARES R and BOURDON R Les formes malignes de l hepatite virale au cours de la gestation Sem Hop Paris 34 1037—1043, 1958

HOYNCK VAN PAPENDRECHT, H P C M Leverfunctiestoornissen in het laatste trimester van de zwangerschap Ned Tijdschr Verlosk en Gynec 57 403—410 1957

HSIA D Y TAYLOR R G and GELLIS S S A long term follow up study on infectious hepatitis during pregnancy J Pediat 41 13—17 1952

*** IKONEN E Jaundice in late pregancy Acta
* obstet gynec Scand 43 Suppl 5 1—130
° 1964
+

IMPARATO E Gli itteri in gravidanza Rass int Clin Therap 38 557—558 1958

INGERSLEV M and TEILUM G Biopsy studies on the liver in pregnancy I Normal histological features of the liver as seen on aspiration biopsy Acta obstet gynec Scand 25 339—351 1945

INGERSLEV M and TEILUM G Biopsy studies on the liver in pregnancy II Liver biopsy on normal pregnant women Acta obstet gynec Scand 25 352—360 1945

INGERSLEV M and TEILUM G Biopsy studies on the liver in pregnancy III Liver biopsy in albuminuria of pregnancy eclampsism and eclampsia Acta obstet gynec Scand 25, 361—376 1945

INGERSLEV M and TEILUM G Jaundice during pregnancy Acta obstet gynec Scand 31, 74—89, 1951

JAVERT, C T and MORRISON R C Jaundice II Its relationsship to pregnancy Texas J Med 47, 137—141, 1951

JEFFRIES, C H and SLEISENGER M H Acute intrahepatic cholestasis Med Clin N America 44 623—632 1960

** JODKOWSKI, H and CHOJECKA B Nawracaja zoltaczka w przebiegu ciazy Pol Tyk Lek 16 891—893, 1961

JOHN G G and KNUDTSON, K P Chronic idiopathic jaundice two cases occurring in siblings, with histochemical studies Amer J Med 21 138—142 1956

+ JUSTIN BESANÇON L PEQUINOT H ETIENNE J P and PHILBERT, M L ictere gravidique recidivant Rev med Franç 40 75—83 1959

KAHIL M E FRED H L BROWN H and DAVIS J S Acute fatty liver of pregnancy Report of two cases Arch Int Med 113 63—69 1964

KALK H Ueber die posthepatitische Hyperbilirubinamie Gastroenterologia 84 207—225 1955

KASDON S C Abdominal pruritus in pregnancy Amer J Obstet Gynec 65 320—324 1953

** KATZ R VELASCO M MANZUR F and
° DONOSO S Ictericia del embarazo Rev Med Chile 89 110—115 1961

KAUFMANN C Ueber die Schwangerschaftsleber Z Geburtsh Gynak 99 582—588 1931

KAUFMANN C Ueber die Schwangerschaftsumstellung der Leberfunktion Klin Wschr 11 493—495 1932

* KEHRER E Zur Lehre von der embryogenen Toxaemia gravidarum Klin Vortrage N F Nr 398 Serie 15 365—404 1905

KEHRER E Die Bedeutung des Ikterus in der Schwangerschaft fur Mutter und Kind Klinische und experimentelle Untersuchungen Arch Gynaec 81 129—159 1907

KELLOG C S and WESP J E Infectious hepatitis during pregnancy and its effect upon the fetus Amer J Obstet Gynec 62 1153—1156 1951

° MYHRE, J. Idiopathic jaundice of pregnancy. Report of a case. Amer J Dig Dis (NS) 8 852—853 1963

NABRISKI S. ZELOOF D. FLEISHMAN P. and LEWITUS Z. Pregnancy in cirrhosis of the liver. J Obstet Gynaec. Brit Emp 65 462—464 1958

NARDONE, A. A. STROUP, P. E. and GILL, R. J. Acute fatty metamorphosis of the liver in pregnancy. Report of a case complicating toxemia in pregnancy. Amer J Obstet Gynec. 80, 258—262 1960

* NASON E. N. Recurring jaundice in mother and infant. Brit. med. J. 1 989—990 1910

NIESERT W. Die Eisenbindungskapazität in der Schwangerschaft. Arch. Gynak. 191 291—296 1958

° NIXON W. C. W. EGELI E. S. LAQUEUR, + W. and YAHYA, O. Icterus in pregnancy. A clinico-pathological study including liver biopsy. J. Obstet. Gynaec. Brit. Emp. 54 642—652 1947

NURNBERGER L. Zur Kenntnis der Leberfunktion am Ende der normalen Schwangerschaft. Arch. Gynaek. 153 1—25 1933

OBER W. O. and LECOMTE P. M. Acute fatty metamorphosis of the liver associated with pregnancy. A distinctive lesion. Amer J. Med. 19 743—758 1955

O CONNELL, W. T. Hepatitis complicating pregnancy. Amer. J. Obstet. Gynec. 63 449—451 1952

O LEARY J. A. and BEPKO F. J. Portocaval shunt performed during pregnancy. Report of a case. Obstet. Gynec. 20 243—246 1962

* ORELLANA J. M. CONZALEZ RODRIGUEZ ° A. and AGUIRRE E. Ictericia benigna del embarazo (Colostasis intrahepática del embarazo) Rev. med. Chile 89 676—679 1961

* ORELLANA J. M. and OSORIO R. Intrahe° patic cholestasis of pregnancy. IInd World Congr. Gastroent. Munich 1962 III 234—238 1963

* PASCHKIS K. Zur Frage des Terrain hépatique. Wien. klin. Wschr. 36 581—581 1923

PAUL, W. M. Infectious hepatitis in pregnancy. Report of a case. Obstet. Gynec. 6 107—110 1955

* PAVEL, I. CEAUSI G. and GARTENBERG A. L'ictere recidivant au cours des grossesses successives. Rev. int. Hepat. 7 317—324 1957

PECK, O. C. REY D. F. and SNELL, A. M. Familial jaundice with free and conjugated bilirubin in the serum and without liver pigmentation. Gastroenterology 39 625—627 1960

* PEL, cited in Vignes, H. p. 47

PERETZ, A. PALDI E. BRANDSTAEDTER, S. and BARZILAI D. Infectious hepatitis in pregnancy. Obstet. Gynec. 14 435—441 1959

* PERREAU P. Ictere recidivant de la grossesse. Arch. mal. appar. digest. 42 1394—1397 1953

** PERREAU P. and ROUCHY R. Ictere * cholostatique recidivant de la grossesse. + Gynec. Obstét. (Paris) 60 161—179 1961

PERREAU P. L'ictere cholostatique recidivant de la grossesse in «Les icteres» Actualités hép. gastroent. hop. Dieu. Masson et Cie. Paris 1962 pp. 224—239

PETERS R. L. EDMONDSON H. A. and KUNELIS C. T. Acute fatty metamorphosis of the liver in pregnancy (Abstract) J. Amer. Med. Ass. 180 167—767 1962

PFAU D. Die Differenzierung der Plasmaproteine in der Schwangerschaft durch Elektrophorese. Geburtsh. Frauenheilk. 11 420—424 1951

PHATAK L. V. and PATIL, K. A study of infective hepatitis in pregnancy. Indian J. Med. Sci. 10 593—601 1956

*** PIERAGNOLI E. D'ANTUONO G. and TURA, S. Ittero recidivante gravidico. Giorn. Clin. Med. 41 516—523 1960

PIRNOLI M. Hepatitis a virus y embarazo. Semana med. 64 1304—1309 1957

POPPER, H. and SCHAFFNER F. Pathology of jaundice resulting from intrahepatic cholestasis. J. Amer. Med. Ass. 169 1447—1453 1959

POPPER, H. RUBIN E. and SCHAFFNER, F. The problem of primary biliary cirrhosis. Editorial. Amer. J. Med. 33 807—810 1962

McNair, R D and Jaynes, R V Alterations in liver function during normal pregnancy Amer J Obstet Gynec *80* 500—505, 1960

* Magnani L Sull ittero recidivante della gravidanza Riv ital Ginec 9, 801—816, 1929

Mansell R V Infectious hepatitis in the first trimester of pregnancy and its effect on the fetus Amer J Obstet Gynec *69*, 1136—1139 1955

Martin, R and Ferguson, F C Infectious hepatitis associated with pregnancy A report of four cases New Engl J Med *237*, 114—117 1947

Martini, G Hepatitis und Schwangerschaft Schweiz Z Allg Path *16* 475—482, 1953

Martini G A von Harnack, G A and Napp J H Hepatitis und Schwangerschaft Die Auswirkung der Hepatitis auf die Mutter Dtsch med Wschr *78*, 661—665 1953

Mason D G Acute fatty metamorphosis of the liver associated with pregnancy (C P C) Northwest Med *57* 456—462 1958

Mason, J M and Wróblewski F Serum glutamic oxalacetic transaminase activity in experimental and disease states A review Arch Int Med *99* 245—252 1957

* Mayer, A Schwangerschaftsikterus als echte eingeschlechtliche Krankheit Med Klinik *2* 1169—1172 1906

Mazad, R Moissinac J and Labegorre J L hépatite ictérigène de la grossesse Méd trop (Marseille) *19* 7—22, 1959

* Meeroff M Ictericias del embarazo Prensa med Argent *48* 3313—3320, 1961

Meinhold Ein weiterer Fall von Schwangerschaftshamoglobinurie Munch med Wschr *50* 166—166 1903

Meranze T Meranze D R and Rothman M M Blood phosphatase in pregnancy Amer J Obstet Gynec *33* 444—450 1937

Merletti C Urobilinurie bei Schwangeren und Vermehrung derselben in Fallen endouterinen Fruchttodes Zbl Gynak *26* 417—421 1902

Metzger, L Zur Casuistik des menstruellen Ikterus Z klin Med *53*, 149—152, 1904

Meulengracht E A review of chronic intermittent juvenile jaundice Quart J Med *16* 83—98, 1947

Meunier J Essai critique sur l ictere des femmes enceintes à propos de l epidemie de Paris 1871—1872, These, Paris 1862 p 17

* Meyer J Ikterus und Schwangerschaft Geburtsh Frauenheilk *15*, 1013—1019, 1955

Meyer M L A Ictere de la grossesse Semaine med *27* 32—32, 1907

Mickal, A Infectious hepatitis in pregnancy Amer J Obstet Gynec *62* 409—414 1951

Millar D The significance of jaundice in pregnancy J Obstet Gynaec Brit Emp *61* 405—405 1954

Millen R M Jaundice during pregnancy Brit J Clin Pract. *11* 341—345 1957

Moore H C Acute fatty liver of pregnancy Irish J Med Sci *6* 451—458 1955

Moore H C Acute fatty liver of pregnancy J Obstet Gynaec Brit Emp *63* 189—198 1956

*** Moore H C Recurrent jaundice of pregnancy A type of intrahepatic cholestasis
**
° Lancet *2* 57—59 1963

Moore, R M and Hughes P K Cirrhosis of the liver in pregnancy A review of the literature and report of three cases Obstet Gynec *15*, 753—756 1960

Moore W J Infectious hepatitis and pregnancy Obstet Gynec *16* 693—698 1960

Moyer, J H Kinross Wright V and Finney R M Chlorpromazine as a therapeutic agent in clinical medicine Arch Int Med *95* 202—218 1955

Mukherjee C Plasma alkaline phosphatase in toxaemia of pregnancy J Indian Med Ass *21* 43—52 1951

° Muller S and Felsch G Hepatitis und Hepatose in der Schwangerschaft Med Mschr *17* 300—305 1963

* Mullerheim, H Diskussion zu Schaeffer O Zbl Gynak *26* 1125—1125 1902

Munnell, E W and Taylor, H C Liver blood flow in pregnancy — hepatic vein catheterization J Clin Invest 29 952—956 1947

jugated bilirubin in the serum A case study New Engl J Med *260* 1315—1318 1959

SCHMID M HEFTI M L GATTIKER R KISTLER, H J and SENNING A Postoperative intrahepatic cholestasis — a benign postoperative jaundice New Engl J Med *272* *545* *550* *1965*

\+ SCHNEIDER W and FRAHM H Beitrag zur hamolytischen Krise und zur perniciosa ahnlichen Anamie in der Schwangerschaft bei familiärem hamolytischem Ikterus Dtsch med Wschr *85* 2074—2076 1960

SCHUBERT R and PETERS H Ikterus und Graviditat unter besonderer Berucksichtigung der Virushepatitis Medizinische *23* 315—318 1954

SCHULTZ J C ADAMSON J S WORKMAN W W and NORMAN T D Fatal liver disease after intravenous administration of tetracycline in high dosage New Engl J Med. *268* 999—1004 1963

* SCHWALM M Recidivierender Schwangerschaftsikterus Zbl Gynak *56* 2098—2105 1932

SEITZ L Schwangerschaftsikterus in Doderlein A Handbuch der Geburtshilfe Verlag J F Bergmann Wiesbaden 1916 Band II p 215—217

SEITZ, L Schwangerschaftshepatocholepathien in Stoeckel W Lehrbuch der Geburtshilfe 10 Auflage Gustav Fischer Jena 1948 p 539—541

SENATOR H Ueber menstruelle Gelbsucht Berl klin Wschr *9* 615—618 1872

** SEYDL G Der Ikterus in der Schwangerschaft Medizin heute *11* 528—531 1962

SHEEHAN H L The pathology of hyperemesis and vomiting of late pregnancy J Obstet Gynaec Brit Emp *46* 685—699 1939

SHEEHAN H L The pathology of acute yellow atrophy and delayed chloroform poisoning J Obstet Gynaec Brit Emp *47* 49—62 1940

* SHEEHAN H L Jaundice in pregnancy Amer J Obstet Gynec *81* 427—440 1961

SHERLOCK S Diseases of the liver and biliary system Blackwell Oxford 3rd ed 1963 pp 305—307 491—495

SIEGAL, I A Liver function in pregnancy Amer J Obstet. Gynec. *14* 300—312 1927

SIEGLER, A. M and KEYSER, H Acute hepatitis in pregancy a report of ten cases and review of the literature Amer J Obstet Gynec. *86* 1068—1073, 1963

** *SIMMONS, S C Recurrent jaundice in pregnancy* Lancet *2* 60—61 1963

SLATER, R J Investigation of an infant born of a mother suffering from cirrhosis of the liver Pediatrics *13* 308—316 1954

SLAUGHTER C. R. and KRANTZ, K E. Cirrhosis of the liver complicating pregnancy a presentation of 2 cases and a review of the literature Amer J Obstet Gynec *86* 1060—1067 1963

SOFFER, L. J Bilirubin excretion as a test for liver function during normal pregnancy Bull Johns Hopkins Hosp *52* 365—375 1933

SPEERT H GRAFF S and GRAFF A M Serum phosphatase relations in mother and fetus Amer J Obstet Gynec *59* 148—154 1950

STACEY C H AZIMA, H HUESTIS D W HOWLETT J G and HOFFMAN M M Jaundice occurring during the administration of chlorpromazine Canad Med Assoc J *73* 386—392 1955

STANDER H J and CADDEN J F Acute yellow atrophy of the liver in pregnancy Amer J Obstet Gynec *28* 61—69 1934

STOKES J WOLMAN I J BLANCHARD M C and FARQUHAR J D Viral hepatitis in the newborn clinical features epidemiology and pathology (Abstract) Amer J Dis. Child *82* 213—213 1951

STONE, M L. LENDING M SLOBODY L. B and MESTERN J Glutamic oxalacetic transaminase and lactic dehydrogenase in pregnancy Amer J Obstet. Gynec. *80* 104—107 1960

VON STUDNITZ W Studies on serum lipids and lipoproteins in pregnancy Scand J Clin Lab Invest. *7* 329—335 1955

SULLIVAN C. F TEW W P and WATSON E. M The bilirubin excretion test of liver function in pregnancy J Obstet. Gynaec Brit Emp. *41* 347—368 1934

SUMMERSKILL, W H J and WALSHE, J M

Potter M G Observations of the gallbladder and bile during pregnancy at term J Amer Med Ass *106*, 1070—1074, 1936

Puder, H Die akute Leberinsuffizienz in der Geburtshilfe Munch med Wschr *51* 1713—1715 1955

* Puyo, G Contribution à l étude de certains
o icteres de la grossesse These, Paris 1953
+

Quincke H and Hoppe Seyler G Die Krankheiten der Leber A Holder, Wien 1899 p 133

Quirno, N, Nolting D and Di Fonzo N O Gestosis a forma hepatica Prensa med Argent *35* 2334—2337 1948

Read A E Harrison C V and Sherlock S Chronic chlorpromazine jaundice with particular reference to its relationship to primary biliary cirrhosis Amer J Med *31*, 249—258, 1961

Reichard H Wiqvist N and Yllner S Serum ornithyl carbamyl transferase activity in normal pregnancy and in pregnancy complicated by pruritus Acta obstet gynec Scand *40* 244—251 1961

Richman A The liver in Guttmacher A F and Rovinsky J J pp 187—201

Rimbach E Die funktionelle Leistungsinsuffizienz in der Graviditat mit besonderer Berucksichtigung des nephrotoxischen Schwangerschaftsikterus Geburtsh Frauenheilk *22* 1264—1266 1962

+ Rimbach E and Beickert A Hamolytischer Ikterus in der Schwangerschaft Munch med Wschr *97* 876—877 1955

Rimbach E and Bonow A Aenderungen der Transaminaseaktivitat wahrend der Schwangerschaft und im Wochenbett Dtsch med Wschr *84* 1822—1825 1959

Rissmann P Langdauernder Steinverschluss des Choledochus und des Diverticulum Vateri — transduodenale Operation in der Graviditat Zbl Gynaek *33* 689—691 1909

+ Rissmann P Gibt es eine den Frauen eigentumliche Form der Gelbsucht? Eine literarisch klinische Studie Z Geburtsh Gynak *65* 325—335 1910

Rissmann, P Milz und Leber in ihren Beziehungen zu den Stoffwechselstorungen der Schwangerschaft Zbl Gynak *41*, 641—643, 1917

Robertson, H E and Dochat, G R Pregnancy and gallstones A collective review Internat Abstr Surg *78* 193—204 1944

* Rolleston, H D Remarks on a case of recurring jaundice in four successive pregnancies, with fatal jaundice in three successive infants Brit Med J *1* 864—865, 1910

Roth, L G Infectious hepatitis in pregnancy Amer J Med Sci *225* 139—146 1953

Salmon G W and Richman, E E Liver function in the newborn infant J Pediat *23* 522—533 1943

Samuels B Jaundice in pregnancy A 10-year review at Touro infirmary Obstet Gynec. *17* 103—108, 1961

Sanders M Hematologic complications in Guttmacher, A F and Rovinsky J J pp 396—413

Saurer A Hepatitis epidemica in Graviditat und Wochenbett. Besondere Bedeutung einiger Beobachtungen fur die Frauenheilkunde und interne Medizin Mschr Geburtsh Gynak *115* 16—33 1943

Scaglione S Cirrosi di Laennec in gravidanza Rev ital Ginec *1* 489—496 1923

+ Schaeffer, O Ein Beitrag zur Aetiologie des wiederkehrenden Ikterus gravidatis Mschr Geburtsh Gynak *15* 897—920 1902

Schaffner F Iatrogenic jaundice J Amer Med Ass *174* 1690—1695 1960

Schapiro R H and Isselbacher K J Benign recurrent intrahepatic cholestasis New Engl J Med *268* 708—711 1963

Schickele G Beitrage zur Physiologie und Pathologie der Schwangerschaft (Schwangerschaftsleber Atypische Eklampsie) Arch Gynak *92* 374—465 1910

Schickele G Neuere Untersuchungen uber die sogenannte Schwangerschaftsleber (hepatotoxemie gravidique) Zbl Gynak *36* 1325—1325 1912

Schiff L Billing B H and Oikawa Y Familial non hemolytic jaundice with con

jugated bilirubin in the serum A case study New Engl J Med. 60 1315—1318 1959
Schmid M Hefti M L Gattiker R Kistler H J and Senning A Postoperative intrahepatic cholestasis — a benign postoperative jaundice New Engl J Med 272 545-550 1965
+ Schneider, W and Frahm H Beitrag zur hamolytischen Krise und zur perniciosa ähnlichen Anamie in der Schwangerschaft bei familiärem hamolytischem Ikterus Dtsch. med Wschr 85 2074—20 6 1960
Schubert R and Peters H Ikterus und Graviditat unter besonderer Berucksichtigung der Virushepatitis. Medizinische 73 315—318 1954
Schultz J C. Adamson J S Workman W W and Norman T D Fatal liver disease after intravenous administration of tetracycline in high dosage New Engl J Med. 268 999—1004 1963
* Schwalm M Recidivierender Schwangerschaftsikterus. Zbl Gynak. 56 2098—2105 1932
Seitz L Schwangerschaftsikterus in Doderlein A Handbuch der Geburtshilfe Verlag J F Bergmann Wiesbaden 1916 Band II p 215—217
Seitz L Schwangerschaftshepatocholepathien in Stoeckel W Lehrbuch der Geburtshilfe 10 Auflage Custav Fischer Jena 1948 p 539—541
Senator H Ueber menstruelle Gelbsucht Berl klin Wschr 9 615—618 1872
** Seydl G Der Ikterus in der Schwangerschaft Medizin heute 11 528—531 1962
Sheehan H L. The pathology of hyperemesis and vomiting of late pregnancy J Obstet Gynaec Brit. Emp 46 685—699 1939
Sheehan H L The pathology of acute yellow atrophy and delayed chloroform poisoning J Obstet Gynaec Brit. Emp 47 49—62 1940
Sheehan H L Jaundice in pregnancy Amer J Obstet Gynec 81 427—440 1961
Sherlock S Diseases of the liver and biliary system Blackwell, Oxford 3rd ed 1963 pp 305—307 491—495
Siegal, I A Liver function in pregnancy Amer J Obstet Gynec 14 300—312 1927
Siegler, A M and Keyser, H Acute hepatitis in pregnancy a report of ten cases and review of the literature Amer J Obstet Gynec 86 1068—1073 1963
** Simmons S C. Recurrent jaundice in pregnancy Lancet 2 60—61 1963
Slater R. J Investigation of an infant born of a mother suffering from cirrhosis of the liver Pediatrics 13 308—316 1954
Slaughter C R and Krantz K E Cirrhosis of the liver complicating pregnancy a presentation of 2 cases and a review of the literature Amer J Obstet Gynec 86 1060—1067 1963
Soffer, L J Bilirubin excretion as a test for liver function during normal pregnancy Bull Johns Hopkins Hosp 52 365—375 1933
Speert H Graff S and Graff A M Serum phosphatase relations in mother and fetus. Amer J Obstet Gynec. 59 148—154 1950
Stacey C H Azima H Huestis D W Howlett J G and Hoffman M M Jaundice occurring during the administration of chlorpromazine Canad Med Assoc J 73 386—392 1955
Stander, H J and Cadden J F Acute yellow atrophy of the liver in pregnancy Amer J Obstet Gynec 28 61—69 1934
Stokes J Wolman I J Blanchard M C and Farquhar J D Viral hepatitis in the newborn clinical features epidemiology and pathology (Abstract) Amer J Dis Child 82 213—213 1951
Stone M L. Lending M Slobody L. B and Mestern J Glutamic oxalacetic transaminase and lactic dehydrogenase in pregnancy Amer J Obstet Gynec 80 104—107 1960
von Studnitz, W Studies on serum lipids and lipoproteins in pregnancy Scand J Clin Lab Invest. 7 329—335 1955
Sullivan C. F Tew W P and Watson E. M. The bilirubin excretion test of liver function in pregnancy J Obstet Gynaec Brit. Emp 41 347—368 1934
Summerskill, W H J and Walshe J M

POTTER M G Observations of the gallbladder and bile during pregnancy at term J Amer Med Ass *106*, 1070—1074 1936

PUDER, H Die akute Leberinsuffizienz in der Geburtshilfe Munch med Wschr *51*, 1713—1715 1955

* PINO, G Contribution à l'étude de certains ° ictères de la grossesse These, Paris 1953
+

QUINCKE, H and HOPPE SEYLER G Die Krankheiten der Leber A Holder, Wien 1899, p 133

QUIRNO, N, NOLTING, D and DI FONZO, N O Gestosis a forma hepatica Prensa med Argent *35*, 2334—2337, 1948

READ A E, HARRISON C V and SHERLOCK, S Chronic chlorpromazine jaundice with particular reference to its relationship to primary biliary cirrhosis Amer J Med *31* 249—258, 1961

REICHARD H WIQVIST, N and YLLNER S Serum ornithyl carbamyl transferase activity in normal pregnancy and in pregnancy complicated by pruritus Acta obstet gynec Scand *40* 244—251 1961

RICHMAN A The liver in Guttmacher A F and Rovinsky J J pp 187—201

RIMBACH E Die funktionelle Leistungsinsuffizienz in der Graviditat mit besonderer Berucksichtigung des nephrotoxischen Schwangerschaftsikterus Geburtsh Frauenheilk *22* 1264—1266, 1962

+ RIMBACH E and BEICKERT A Hamolytischer Ikterus in der Schwangerschaft Munch med Wschr *97* 876—877 1955

RIMBACH E and BONOW A Aenderungen der Transaminaseaktivitat wahrend der Schwangerschaft und im Wochenbett Dtsch med Wschr *84* 1822—1825 1959

RISSMANN P Langdauernder Steinverschluss des Choledochus und des Diverticulum Vateri — transduodenale Operation in der Graviditat Zbl Gynaek *33* 689—691 1909

+ RISSMANN P Gibt es eine den Frauen eigentumliche Form der Gelbsucht? Eine literarisch klinische Studie Z Geburtsh Gynak *65* 325—335 1910

RISSMANN, P Milz und Leber in ihren Beziehungen zu den Stoffwechselstorungen der Schwangerschaft Zbl Gynak *41*, 641—643, 1917

ROBERTSON, H E and DOCHAT G R Pregnancy and gallstones A collective review Internat Abstr Surg *78* 193—204 1944

* ROLLESTON, H D Remarks on a case of recurring jaundice in four successive pregnancies with fatal jaundice in three successive infants Brit Med J *1*, 864—865 1910

ROTH, L G Infectious hepatitis in pregnancy Amer J Med Sci *225* 139—146, 1953

SALMON, G W and RICHMAN F E Liver function in the newborn infant J Pediat *23*, 522—533 1943

SAMUELS B Jaundice in pregnancy A 10-year review at Touro infirmary Obstet Gynec *17* 103—108 1961

SANDERS M Hematologic complications in Guttmacher A F and Rovinsky J J pp 396—413

SAURER A Hepatitis epidemica in Graviditat und Wochenbett Besondere Bedeutung einiger Beobachtungen für die Frauenheilkunde und interne Medizin Mschr Geburtsh Gynak *115* 16—33 1943

SCAGLIONE S Cirrosi di Laennec in gravidanza. Rev ital Ginec *1* 489—496 1923

+ SCHAEFFER O Ein Beitrag zur Aetiologie des wiederkehrenden Ikterus graviditatis Mschr Geburtsh Gynak *15* 897—920 1902

SCHAFFNER F Iatrogenic jaundice J Amer Med Ass *174* 1690—1695 1960

SCHAPIRO R H and ISSELBACHER K J Benign recurrent intrahepatic cholestasis New Engl J Med *268* 708—711 1963

SCHICKELE G Beitrage zur Physiologie und Pathologie der Schwangerschaft (Schwangerschaftsleber Atypische Eklampsie) Arch Gynak *92* 374—465 1910

SCHICKELE G Neuere Untersuchungen uber die sogenannte Schwangerschaftsleber (hépatotoxemie gravidique Zbl Gynak *36* 1325—1325 1912

SCHIFF L BILLING B H and OIKAWA Y Familial non hemolytic jaundice with con

jugated bilirubin in the serum A case study New Engl J Med. 260 1315—1318 1959
SCHMID M HEFTI M L. GATTIKER, R KISTLER, H J and SENNING A. Postoperative intrahepatic cholestasis — a benign post operative jaundice New Engl J Med 272 545 550 1965
* SCHNEIDER W and FRAHM H Beitrag zur hamolytischen Krise und zur perniciosa ahnlichen Anamie in der Schwangerschaft bei familiarem hamolytischem Ikterus. Dtsch med Wschr 85 2074—2076 1960
SCHUBERT R and PETERS H Ikterus und Graviditat unter besonderer Berucksichtigung der Virushepatitis Medizinische 23 315—318 1954
SCHULTZ J C. ADAMSON J S WORKMAN W W and NORMAN T D Fatal liver disease after intravenous administration of tetracycline in high dosage New Engl. J Med 268 999—1004 1963
* SCHWALM M Recidivierender Schwangerschaftsikterus. Zbl Gynak 56 2098—2105 1932
SEITZ L Schwangerschaftsikterus in Doderlein A Handbuch der Geburtshilfe Verlag J F Bergmann Wiesbaden 1916 Band II p 215—217
SEITZ L Schwangerschaftshepatocholepathien in Stoeckel W Lehrbuch der Geburtshilfe 10 Auflage Gustav Fischer Jena 1948 p 539—541
SENATOR H Ueber menstruelle Gelbsucht Berl klin Wschr 9 615—618 1872
** SEYDL G Der Ikterus in der Schwangerschaft Medizin heute 11 528—531 1962
SHEEHAN H L The pathology of hyperemesis and vomiting of late pregnancy J Obstet Gynaec Brit. Emp 46 685—699 1939
SHEEHAN H L The pathology of acute yellow atrophy and delayed chloroform poisoning J Obstet Gynaec Brit. Emp 47 49—62 1940
* SHEEHAN H L Jaundice in pregnancy Amer J Obstet Gynec 81 427—440 1961
SHERLOCK, S Diseases of the liver and biliary system Blackwell Oxford 3rd ed 1963 pp 305—307 491—495
SIEGAL, I A Liver function in pregnancy Amer J Obstet Gynec. 14 300—312 1927
SIEGLER, A M and KEYSER, H Acute hepatitis in pregancy a report of ten cases and review of the literature Amer J Obstet Gynec. 86 1068—1073 1963
** SIMMONS S C. Recurrent jaundice in pregnancy Lancet 2 60—61 1963
SLATER, R J Investigation of an infant born of a mother suffering from cirrhosis of the liver Pediatrics 13 308—316 1954
SLAUGHTER, C. R. and KRANTZ K. E. Cirrhosis of the liver complicating pregnancy a presentation of 2 cases and a review of the literature Amer J Obstet. Gynec. 86 1060—1067 1963
SOFFER L. J Bilirubin excretion as a test for liver function during normal pregnancy Bull Johns Hopkins Hosp 52 365—375 1933
SPEERT H GRAFF S and GRAFF A. M Serum phosphatase relations in mother and fetus. Amer J Obstet Gynec. 59 148—154 1950
STACEY C H AZIMA, H HUESTIS D W HOWLETT J G and HOFFMAN M M Jaundice occurring during the administration of chlorpromazine Canad. Med Assoc J 73 386—392 1955
STANDER, H J and CADDEN J F Acute yellow atrophy of the liver in pregnancy Amer J Obstet. Gynec. 28 61—69 1934
STOKES J WOLMAN I J BLANCHARD M C and FARQUHAR J D Viral hepatitis in the newborn clinical features, epidemiology and pathology (Abstract) Amer J Dis. Child 82 213—213 1951
STONE M L. LENDING M SLOBODY L. B and MESTERN J Glutamic oxalacetic transaminase and lactic dehydrogenase in pregnancy Amer J Obstet Gynec 80 104—107 1960
VON STUDNITZ, W Studies on serum lipids and lipoproteins in pregnancy Scand J Clin Lab Invest 7 329—335 1955
SULLIVAN C. F TEW W P and WATSON E M The bilirubin excretion test of liver function in pregnancy J Obstet Gynaec. Brit. Emp 41 347—368 1934
SUMMERSKILL, W H J and WALSHE J M

Benign recurrent intrahepatic «obstructive» jaundice Lancet *2* 686—690, 1959

** SVANBORG A A study of recurrent jaundice
° in pregnancy Acta obstet gynec Scand *33* 434—444, 1954

*** SVANBORG, A and OHLSSON, S Recurrent
* jaundice of pregnancy A clinical study of
° twenty two cases Amer J Med *27* 40—49, 1959

SYNODINOS E Ictere et grossesse Rev Franç Gynec Obstét *58*, 313—344, 1963

SYNODINOS E Ictere et grossesse Gynec Obstét (Paris) *62* 107—134, 1963

SYNODINOS, E FRANGIADAKIS L., PAPADOYIANAKIS, N and STAVROPOULOS A Ictere et grossesse Rev Med moyen Orient *19*, 84—92 1962

TAMAKI H T and CARFAGNO S C Chronic idiopathic jaundice with unidentified pigment in liver cells Arch Int Med *99* 294—296, 1957

TAYLOR H C Infectious hepatitis in pregnancy Connecticut Med J *16* 587—591, 1952

TENNEY, B and KING, R B Pregnancy coincident with cirrhosis of the liver report of a case New Engl J Med *208* 1157—1160 1933

** THORLING L Jaundice in pregnancy A
° clinical study Acta med Scand Suppl
+ *302* 1—123 1955

TOPP, J R and CHARLES B Pruritus of pregnancy A symptom of hepatic dysfunction with a report of two cases Canad Med Assoc J *85* 724—726 1962

TYGSTRUP N Intermittent possibly familial intrahepatic cholestatic jaundice Lancet *1* 1171—1172 1960

+ TYLECOTE F E Jaundice of pregnancy associated with jaundice in the offspring Med Chronicle 58 465—468 1914

TYSOE F W and LOWENSTEIN L Blood volume and hematologic studies in pregnancy and the puerperium Amer J Obstet Gynec *60*, 1187—1205 1950

° VAN WOERT M H and KIRSNER J B Idiopathic jaundice of pregnancy Gastroenterology *40*, 633—635 1961

VARANGOT, J Les icteres de la grossesse in «Les icteres», Actualites hep gastroent Hop Dieu Masson et Cie Paris *1962*, pp *215—223*

VERHAGE, J C Hyperemesis gravidarum Nederl Tijdschr Verlosk Gynec *43* 36—74 1940

* VERHAGE, J C Icterus in de zwangerschap Nederl Tijdschr Verlosk Gynec *43* 135—159 1940

VERMEHREN E *Plasmaphosphatase wahrend* der Graviditat und der Laktation Acta med Scand *100* 254—266 1939

+ VIGNES H Maladies des femmes enceintes II Affections du foie, du pancreas Maladies de la nutrition Parois abdominale Peritoine Masson et Cie Paris 1935, p 42

VINAY Ch Traite des maladies de la grossesse et des suites de couches *1894* cited in Becking A G T

VINCENT, C R Jaundice in pregnancy A review from the Charity Hospital New Orleans 1941—1956 Obstet Gynec *9* 595—598 1957

* VON DEN VELDEN R Icterus gravidarum Beitr Geburtsh Gynaek *8* 448—464 1904

WATSON C J and HOFFBAUER F W The problem of prolonged hepatitis with particular reference to the cholangiolitic type and to the development of cholangiolitic cirrhosis of the liver Ann Int Med *25* 195—227 1946

WERNER S C HANGER F M and KRITZLER R A Jaundice during methyl testosterone therapy Amer J Med *8* 325—331 1950

WERTHER J L and KORELITZ B Chlorpromazine jaundice Analysis of twenty two cases Amer J Med *2? 351—366 1957*

WEST M and ZIMMERMAN H J Lactic dehydrogenase and glutamic oxaloacetic transaminase in normal pregnant women and newborn children Amer J Med Sci *235 443—447 1958*

WETSTONE H J LA MOTTA R V MIDDLEBROOK L TENNANT R and WHITE B V Studies of cholinesterase activity IV Liver function in pregnancy Values of certain

standard liver function tests in normal pregnancy Amer J Obstet Gynec *76* 480—490 1958

* WEWALKA, F Beitrag zur Hepatitis in der Schwangerschaft Verhandl Deutsch Ges Inn Med (63 Kongress 1957) *63* 392—396 1957

WHALLEY P J ADAMS R. H and COMBES B Tetracycline toxicity in pregnancy Liver and pancreatic dysfunction J Amer Med Ass *189* 357—362 1964

WHITACRE F E and FANG L. Y Fatty degeneration of the liver in pregnancy Report of a case with recovery chemical and histologic studies. J Amer Med Ass *118* 1358—1364 1942

* WILKEN H Zur Frage der Leberschadigung in der Schwangerschaft Dtsch Gesundheitswes. *13* 1432—1435 1958

WILKEN H Die »Leberclearance« mit Bromsulphalein in der Schwangerschaft und unter der Geburt Zbl Gynak *82* 1804—1809 1960

WILLIAMS H M Liver disease in pregnancy Connecticut Med J *21* 497—501 1957

WILLIAMS R. CARTTER M A SHERLOCK S SCHEUER, P J and HILL, K R. Idiopathic recurrent cholestasis a study of the function and pathological lesions in four cases. Quart J Med (N S) *33* 387—399 1964

VON WINCKEL, F Icterus gravidarum Lehrbuch der Geburtshilfe von Keit & Co Leipzig 1893 2 Aufl pp 239—241

WINTER J T Jaundice during pregnancy Amer J Obstet Gynec *23* 31—37 1890

YOUNG J KING E. J WOOD E and WOOTTON I D P A nutritional survey among pregnant women J Obstet Gynaec Brit Emp *53* 251—259 1946

+ ZACHARIAE, F A case of haemolytic anaemia induced by pregnancy Acta obstet. gynec Scand 37 250—263 1953

ZONDEK, B and BROMBERG Y M Infectious Hepatitis in pregnancy J Mt Sinai Hosp *14* 222—243 1947

ACTA MEDICA SCANDINAVICA

SUPPLEMENTUM 445

IN HONOUR OF

JAN WALDENSTRÖM

ON HIS SIXTIETH BIRTHDAY

APRIL 17 1966

Accompanies Vol 179

LUND 1966

ACTA MEDICA SCANDINAVICA

has been published since 1919 as a continuation of Nordiskt Medicinskt Arkiv, founded in 1869 by Axel Key The first volume of Acta Medica Scandinavica is therefore numbered LII (52)

The chief editors have been Axel Key 1869—1900, C G Santesson 1901—1915, I Holmgren 1916—1957 and Birger Strandell 1958 to date

Acta Medica Scandinavica publishes original research papers in the field of internal medicine Priority will be given to authors from countries which are represented on the editorial board Contributors from those countries should submit their papers to a representative of the country in question Contributors from other countries should send their papers to the Editor at the address below

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Subscription

The annual subscription to the journal, covering two volumes, each of 6 numbers is 140 Sw crowns or U S $ 27 25 *including postage* in the Scandinavian countries and in Holland 120 Sw crowns

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ACTA MEDICA SCANDINAVICA
SUPPLEMENTUM 445

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DEDICATED PAPERS ON THE OCCASION
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REDIGENDA CURAVIT
SVEN ERIK BJÖRKMAN

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JAN WALDENSTROM

APRIL 17 1966

Jan Waldenstrom is professor of a medical faculty of the third generation His grandfather Johan Waldenström was professor of internal medicine in Uppsala and his father Henning Waldenström is retired as professor of orthopedic surgery in Stockholm Jan Waldenström, who has become a firm believer in the importance of genetic factors in metabolic diseases is thus himself a *living evidence* that the destiny of man is ruled by his genes

Medicine of today and particularly internal medicine is faced with vast problems Rapid progress has created a state of flux It is impossible to predict or even guess the future course of medicine There is a tendency towards division into smaller and more specialised branches This has created an urgent need for men equipped with receptiveness to the complete multiplicities of human experience coupled with polymathic knowledge of past and modern medical science and at the same time with the ability to set up signposts for new approaches to diagnostic and therapeutic problems

What is required by such a man? A comprehensive and vigilant brain an apprehensive and open scientific mind a broad and keen interest in human behaviour sharpness of observation a selective pigeon holed memory and intrepidity

Jan Waldenström has these qualities — by inheritance During the last thirty years he has entered widely separated fields of medicine to all of which he has contributed with original observations of lasting value His work has greatly influenced not only the research work of his contemporaries but also the march of medicine in general

None the less it would be wrong to think that he has not followed his own line As a young doctor he was given the unique opportunity of studying organic chemistry in the laboratory of Hans Fischer in Munich the pyrrol pigment chemist and Nobel prize winner This biochemical background has

Of utmost importance for these studies was the unique opportunity Waldenström had in Uppsala to collaborate with members of the Institute of Physical Chemistry In collaboration with Pedersen sedimentation constants and electrical mobility of serum proteins in different diseases with elevated LSR were determined by means of The Swedberg's ultracentrifuge and Arne Tiselius electrophoresis method It is not presumptous to state that the foundation stone of the present surge of interest in serum protein disturbances was laid there

Waldenstrom recognized in the early 1950s the qualitative difference between the increased globulins in myeloma and diseases due to chronic infection or collagen states namely the electrophoretically narrow and broad peak gamma type He concluded that in myeloma only a part of the gamma fraction was increased in contrast to the broad gamma peak. This fundamental observation led him logically ten years later to coin the names monoclonal and polyclonal for these hypergammaglobulinemias By that time it had become apparent that there also existed a clinically benign type of narrow peak gamma not associated with myeloma So now the follow up of patients with an elevated ESR proceeds as a follow up of patients with narrow bands in order to settle the clinical importance of this finding In this connection he has also coined the name gammapathies and stressed the importance of hereditary factors in the causation of gamma disorders He infers that the synthesis of gammaglobulins is genetically controlled Another example of this is the change of the gamma synthesis in the opposite direction seen in the hereditary sex linked agammaglobulinemia first described by the master of gammapathies, Jan Waldenström

For 25 years Waldenström has had a never waning interest in the myeloma disease not only from the point of protein disturbance but also with respect to its clinical manifestations cellular morphology prognosis and treatment He stands here as always as a link otherwise often missing between the theoretical laboratory research workshop and the practical clinical work His experience of the biological nature of this malady is eminent and his therapeutic program for cytostatic treatment is probably one of the most promising in malignant disorders

Somebody once stated that the compilation of a new syndrome should be a rather simple task for the experienced clinician In these days of computer analysis it requires perhaps even less effort Waldenström s conception of the

resulted in his adopting a metabolic approach to clinical medicine He feels that every disorder is basically an interplay of biochemical reactions He advocates that it is the diagnostic goal to find out what material it is in the sick body that is in excess or deficient as a result of the metabolic disturbance, analogous to the accumulation of porphobilinogen in the urine in acute porphyria

The first example of his biochemical approach to clinical medicine is his classical monograph »Studien uber Porphyrie» (Uppsala 1937), in which acute porphyria for the first time was shown to be a hereditary disorder of porphyrin synthesis which was manifested biochemically by the excretion of uroporphyrin III in the urine This and other works of his brought order into the controversial state of the porphyrias and clarified our concepts It has awarded him a rank of honour as a pioneer in clinical porphyrin research

With his keen interest in pyrrol chemistry it is not surprising that hematology, particularly iron metabolism, became a new field of research A series of articles on hematological subjects followed his porphyrin works Among those large population studies on the incidence of iron deficiency anemia were pioneer work, clearly before its time He was also one of the first to perform iron balance studies long before isotope techniques had been developed Pulmonary hemosiderosis was first described by him as a new clinical entity and with a peculiar type of iron deficiency Sideropenic dysphagia was reevaluated and the roentgen diagnostic procedure was improved Iron deficiency problems have always since been among his favourite subjects and he was the first to describe symptoms of iron deficiency without anemia

While studying these subjects he also began his research in serum proteins I think that the constellation in Uppsala was particularly favourable for such studies at that time The use of the erythrocyte sedimentation rate (ESR) in clinical medicine became a routine procedure in Sweden in the early 1930s Waldenstroms interest in serum proteins emerged from follow up studies of patients with constantly elevated ESR These patients were mostly members of farmer families from the rural neighbourhood of Uppsala who regularly went to town to do their business and shopping and to have their ESR measured Without their knowledge their initials flew all over the world carrying with them new ideas and names of new disease concepts — purpura hyperglobulinaemica macroglobulinaemia Waldenström liver cirrhosis in young girls

1

PORPHYRIN METABOLISM

carcinoid syndrome, however, exemplifies how an observation of a patient who happened to be seen on a ward round can trigger off the imagination of an erudite clinician and bring to mind a multitude of memories and experiences and lead to the creation of a new syndrome At that moment his brain must have acted as an electronic computer This might also be called clinical intuition and it is commonly agreed upon that Jan Waldenstrom has a clinical imaginative eye The importance of this new syndrome can hardly be overrated It has opened our eyes to a hitherto unknown type of humoral activity of tumor tissue

The portrait of Jan Waldenstrom would not be true without credit to his personal qualities, his alluring touch, his rapid apprehension and association, his intuition and empathy These natural gifts are never so well displayed as when he is a little alarmed, as when faced with difficult problems lecturing to a large audience or discussing controversial questions One will also imagine that he uses his personal charm to fight scientific puzzles

This volume pays tribute to Jan Waldenström, the versatile scientist, the sage physician, the learned teacher and medical philosopher It marks a mile stone, sixty years of life thirty years of intense work in medical research Friends, pupils, collaborators and admirers over the whole globe extend to him on this occasion their heartiest congratulations

Sven Erik Björkman

I

PORPHYRIN METABOLISM

From the Department of Chemical Pathology University College Hospital Medical School London W C 1 England

Porphyrin and Haem Biosynthesis and its Control

By C Rimington

The essentially dynamic nature of the life of an entire organism and also of its constituent cells demands of necessity mechanisms for preserving homeostasis — a balanced order among the multitude of reactions taking place This has been achieved at various levels and in various ways The elaboration of organs in multicellular animals and a physiological division of labour between tissues such as muscle nerve cells gonads etc contribute towards such an end at a gross level of organization and the compartmentalization of intracellular reactions in various organelles carries the process on to the most intimate events of cellular metabolism All this complexity demands highly efficient machinery for coordination

The biosynthesis of the porphyrins and haems present in and essential to every cell offers no exception to the general rule indeed it has been pointed out that the efficiency of this synthesis judged by the relative amounts of porphyrins and their precursors synthesised and excreted per day is of an astonishingly high order The system is nevertheless very adaptable and responsive lowered oxygen tension rapidly induces a haemopoietin mediated polycythaemia and the haemoglobin output from the bone marrow of the dog may increase eight fold under the stress of chronic withdrawal of blood In lower forms of life such as the crustacean *Daphnia* colonies may be observed to become red due to accumulation of haemoglobin under conditions of oxygen deprivation and to return equally rapidly to the non coloured state as a result of aeration (13) The induction or repression of cytochrome synthesis in yeasts (67) and bacteria (41) in response to changes in the oxygen content of the medium is another familiar example Only in some pathological conditions do the regulatory mechanisms appear to break down and to result in gross accumulation and excretion of pigments of the tetrapyrrole series Most of these diseases are genetically determined examples of "inborn errors of metabolism" but in both man and animals similar excesses of porphyrin production have been seen to follow

from poisoning by certain chemicals Such "experimental porphyrias" have been closely studied in the hope of understanding more precisly the biochemical lesions responsible for the natural diseases It is the object of this article to review some of these results and to determine how they illuminate the normal homeostasis of porphyrin and haem production

The biosynthetic pathway through porphyrins to haem

Intensive study during the last decade has established the essential steps of the haem biosynthetic pathway (49 50, 21) Certain enzymic steps still require clarification but it is accepted that the point of departure is the union of glycine and succinyl CoA under the influence of ALA synthetase and pyridoxal phosphate to form δ aminolaevulic acid (ALA), with α amino β ketoadipic acid as a probable short lived intermediate This event takes place within the mitochondria ALA must then diffuse out into the cytoplasm where the next series of reactions takes place ALA dehydratase condenses two molecules of ALA to form porphobilinogen (PBG) the first pyrrolic intermediate and it is to be noted that from this step onwards the pathway is irreversible so that the quantity of ALA undergoing pyrrolic condensation will determine the quantity of porphyrin which ultimately appears Four molecules of porphobilinogen are cyclized with loss of 4 mols of ammonia to produce the first tetrapyrrolic substance uroporphyrinogen III, a derivative of hexahydro porphin The mechanism of this cyclization is not fully understood but two factors are necessary, an enzyme having porphobilinogen as its substrate and a co factor, uroporphyrinogen isomerase, necessary for the production of the type III uroporphyrinogen, and which may be an integral part of the whole "porphobilinogenase" complex

Stepwise partial decarboxylation of uroporphymogen III leads to coproporphyrinogen III then for the next 2 steps coproporphyrinogen III must re enter the mitochondrion Here a "coproporphyrinogenase" brings about decarboxylation of the propionic acid side chains occupying the 2 and 4 positions and oxidation so that vinyl groups result, the product being protoporphyrinogen IX These two steps have not been separated in *in vitro* systems and the course of the reaction is obscure It is noteworthy that no substitute for oxygen as oxidant has so far been found for this *in vitro* system

Insertion of ferrous iron into the tetrapyrrole to form haem is accomplished enzymatically within the mitochondria but for this to take place protoporphyrin must be present in the oxidized porphyrin state and not as the porphyrinogen thus an oxidation step must intervene immediately prior to insertion of iron The synthesis of haem by ferrochelatase (Haem synthetase) preparations however requires iron in the ferrous form Having once been formed within the mitochondria haem presumably diffuses into the

extra mitochondrial space where combination with specific proteins presumably takes place to produce the haemoprotein catalysts

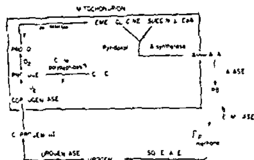


Fig 1 Intracellular location of enzymes participating in haem biosynthesis (Reproduced by permission from Granick and Levere (21))

Importance of compartmentalization of reactions and of oxidation/reduction conditions

It will be evident from this brief summary of the biosynthetic pathway that discrete events demand particular localization and also particular oxidation/reduction conditions of the milieu Alterations in the latter could affect the state of oxidation of thiol groups in the enzymes of the pathway many of which appear to be functional as SH proteins A correct oxidation/reduction milieu may also be necessary not only for maintenance of the tetrapyrroles at the porphyrinogen level in which state they serve as substrates for many of the enzymes but also to satisfy permeability requirements of the various intracellular membranes

This latter point raises another important consideration namely whether the intermediates along the pathway are present in the free state or are protein bound If attached to a large protein molecule which may be necessary for the ensuing enzyme catalysed chemical transformation their velocity of migration will be considerably less than that of the free substance Binding to a protein present in a particular intracellular compartment may also aid concentration within that compartment by preventing back diffusion There is indeed much evidence that some of the porphyrin intermediates serving as substrates for the biosynthetic enzymes do react while bound to proteins Thus Porra and Falk (41) demonstrated covalent attachment of a porphyrin intermediate to protein during the enzymic transformation of coproporphyrinogen III to protoporphyrinogen IX Similarly it appears to be a protein bound form of protoporphyrin which reacts with iron during the enzymic formation of haem (10 40 42) Iron itself is protein bound within the developing red cell (46 11 62) Granick and Levere (21) have summarized our knowledge concerning compartmentalization of the steps occurring in haem biosynthesis in the diagram reproduced as Fig 1

Limitation imposed by availability of substrates

It is perhaps obvious that the production of any substance through a biosynthetic pathway should be limited or subjected to control by the availability of the different materials re

quired for its production Thus iron-deficiency anaemia may result directly from an inadequate supply of iron to the body since iron is an essential constituent of haem and the accumulation of porphyrins by micro organisms cultured under conditions of iron deficiency is also a familiar phenomenon

Porphyrin biosynthesis begins with the union of glycine and succinyl CoA, mediated by the enzyme ALA-synthetase with pyridoxal phosphate as one co factor and possibly ferrous iron as another (3) Deficiency of pyridoxine or pantothenate in the diet can cause an anaemia in animals and man which is corrected by supplying the vitamins (54, 66, 31) Glycine is so readily available in the diet and from intermediary metabolism that a deficiency of this aminoacid is hardly ever likely to occur as a normal event Ycas and Starr (68) have found however, that a *Saccharomyces cerevisiae* mutant grown on minimal medium produced glycine so slowly that synthesis of haem was impaired and the organism contained only low levels of catalase and cytochrome c These levels were raised to normal by addition of either glycine or protoporphyrin IX to the growth medium

Succinyl CoA may be formed in several ways namely (1) from α keto glutarate, derivable from the citric acid cycle, (2) by the direct reaction of succinate with coenzyme A catalysed by a nucleoside triphosphate The latter appears to be either GTP or ITP in animal tissues whereas the enzyme from spinach requires ATP (3) from propionyl CoA (especially in ruminants) which combines with CO_2 in the presence of a carboxylating biotin containing enzyme to give methylmalonyl CoA This latter is converted to succinyl CoA by a B_{12} containing enzyme, (4) through the action of a CoA transferase on acetoacetyl CoA In developing erythrocytes the most important pathway appears to be number 1 although number 2 is also operative Thus Shemin and Kumin (56) blocked succinate oxidation in intact duck erythrocytes by the addition of malonate so that very little α ketoglutarate would be generated from the citric acid cycle When methylene labelled succinate was now added to the system, incorporation of the label into haem took place to an extent suggesting that some 30—50 % of the required succinyl CoA could be derived directly from succinate In line with this conclusion is the finding (3) that addition of ATP to haemolyzed chicken erythrocytes enhanced ALA synthesis only when succinate was the substrate In the whole animal, inhibition of succinic dehydrogenase by administration of fairly large amounts of malonate has been shown recently (35) to increase the excretion of porphyrins and porphyrin precursors

Pathway No 1 is the route for succinyl CoA synthesis most likely to be affected by events in general intermediary metabolism A closer inspection of this pathway in pig heart has revealed (38) that it includes several reaction sequences involving the lipo thiamide system much as originally suggested by Rimington (47 48) These are set forth below

(a) α KG + Thiamine pyrophosphate (TPP) → succinyl TPP + CO

(b) succinyl TPP + S|S>lipoyl enzyme ⇌ succinyl S/HS>lipoyl enzyme + TPP

(c) succinyl S/HS>lipoyl enzyme + CoA SH ⇌ succinyl S CoA + HS/HS>lipoyl enzyme

(d) HS/HS>lipoyl enzyme + S|S>FAD enzyme ⇌ S|S>lipoyl enzyme + HS/HS>FAD enzyme

(e) HS/HS>FAD enzyme + NAD^+ ⇌ S|S>FAD enzyme + NADH + H^+

The integrity of all these systems is essential for the biosynthetic supply of succinyl CoA to proceed smoothly In particular it is necessary that oxygen be freely available for the operation of the cycle

Effect of oxygen tension on biosynthesis of porphyrins

The effect of oxygen tension on the cytochrome and haemoglobin production of whole organisms has already been mentioned briefly and the fact that hormonal adjustments are often brought into play

The tension of oxygen in the suspending medium also appears to have an important effect however on the individual enzymes participating in the haem biosynthetic pathway *in vitro* Whilst such *in vitro* effects upon isolated systems are not necessarily a true indication of the state of affairs in the organized living cell they may nevertheless have some significance in helping us to understand the complexities of *in vivo* control Falk et al (12) have used chick blood or washed erythrocytes with glycine as substrate for such experiments and have shown that coproporphyrin production is maximal at about 1 % oxygen whilst the optimum for protoporphyrin and haem is in the region of 7 % oxygen their formation being markedly depressed at higher oxygen tensions Depression of protoporphyrin synthesis can be reversed by again lowering oxygen tension during the course of the incubation

Control by end product inhibition

It has become apparent in recent years that the activity of several different biosynthetic pathways may be controlled by negative feed back inhibition the end product of the pathway exerting an inhibitory action on the initial enzyme system Such regulation is economical in that it prevents synthesis of the end product over and above the actual requirements of the cell or body as a whole

Such phenomena have been studied mainly in bacteria but we do know from the early work of Whipple (64) that the bone marrow of the dog is capable of increasing its rate of synthesis of haemoglobin by some eight fold under conditions of severe anae

mia. Nevertheless, in the normal homeostatic state the balance sheet of pigment production and excretion by man shows an astonishing degree of efficiency, approximately 460 μ moles of haemoglobin being synthesised per day with a "wastage" of only about 24 μ moles of porphyrin precursors and of less than 0.2 μ moles of porphyrins in the urine (33). Similarly the efficiency of the enzymic system which directs porphobilinogen to uroporphyrinogen III rather than the I series isomer has been calculated (21) to be of the order of 99.9 %.

That tetrapyrrole synthesis in bacteria is controlled by negative feed back inhibition of enzymes of the pathway is suggested by the accumulation of porphyrins occurring under conditions of iron deficiency and in the case of *Rhodopseudomonas spheroides* this phenomena has been studied in detail by Lascelles (30). The quantity of porphyrins formed without iron is about one hundred times greater than the amount of total tetrapyrroles (haems and bacteriochlorophyll) formed with iron. The excreted porphyrins therefore represent over production of an intermediate as a result of the failure of a control mechanism. The action of iron is also catalytic, some 100 μm moles of coproporphyrin being prevented from appearing by only 2 μm moles of iron citrate. Evidence that the actual inhibitor is a compound of iron, viz. haem, and that it acts upon the initial enzyme of the pathway follows from the observations that porphyrin accumulation by intact cells is inhibited by haem when α-ketoglutarate and glycine are the substrates, but not with ALA as substrate, and that the isolated ALA synthetase system is extremely sensitive to haemin, being inhibited significantly by 0.1 μM of haemin. This inhibition is non competetive with any of the substrates or co-factors of the synthetase. So far this negative feed back inhibition of ALA-synthetase by haem has only been established conclusively in the case of *R. spheroides* but it may well operate more widely (26). It may be mentioned at this point that, as pointed out by Lascelles (33) it is always coproporphyrin and not protoporphyrin which accumulates under conditions of iron deficiency, a fact suggesting that iron may play a part in the conversion of coproporphyrinogen to protoporphyrinogen. In the most recent work on this transformation Batlle, Benson and Rimington (1) have observed that their highly purified enzyme developed a red colour on addition of α α' bipyridyl o-phenanthroline or 8-hydroxyquinoline; some degree of inhibition of the transformation was exerted by the first two compounds but none by 8-hydroxyquinoline, sodium azide, potassium cyanide or desferal in the concentrations used. Whether or not some peculiar form of iron linkage is present remains a matter for conjecture.

Control by end-product repression

Apart from the possibility of a degree of control by end product inhibition through the operation of a negative feed back in which the end product

inhibits the initial enzyme involved in its formation biosynthetic systems appear to be regulated mainly by a more complex machinery involving the genetic material of the cell This is termed control by end product repression and our knowledge of it has been derived almost entirely from bacteria The theory originated by Jacob and Monod, (27 28) may be stated in its simplest form with definition of terms as follows a *structural gene* a segment of chromosomal DNA carries the information for a particular enzymatic protein This it imparts to a short lived type of RNA messenger RNA which *in turn carries the information* to the ribosomes where synthesis of the protein takes place Synthesis of messenger RNA is initiated by another region of DNA designated the *operator gene* Structural gene and operator gene together make up an *operon*

Should there be no restraint upon this system continuous uncontrolled production of messenger RNA and of the specific protein would result This is avoided by the action of yet another segment of DNA a *regulator gene* producing a *repressor* which has the ability to block reversibly the activity of its operator gene The repressor in turn may be rendered inactive by *combination* reversibly with an *effector* Conversely an effector may enter into combination with an aporepressor the combination having repressor activity The effectors would appear to be relatively small molecules less complex than proteins Normally therefore biosynthesis of an enzyme protein like ALA synthetase is held firmly in control by its regulator gene and repressor Products of one biosynthetic *chain may act as effectors* in other synthetic processes, so coordinating different pathways Effector activity may also be a property of some hormones e g oestrogen which are able to stimulate protein synthesis *in vivo* (24)

The theory which in its fully developed form is very complex is nevertheless able to explain such phenomena as enzyme induction in bacteria and the segregations observed and predictable from bacterial genetics Its main points have been *recently summarized* by Brenner (2) The properties required of repressors are just those provided by allosteric proteins binding of an inducer molecule at one site being able to reduce the affinity of another site for the operator This may be achieved by deformation charge alteration or otherwise

Formation of ALA synthetase in growing cultures of *R spheroides* is repressed by addition of haemin in concentrations as low as 0 01 mM[32] and this repression appears to be specific since other porphyrins or metal complexes are devoid of activity It is of interest that haemin also exerts repression upon ALA dehydratase the next enzyme in the biosynthetic sequence this being an example of coordinate repression a phenomenon often witnessed in bacterial biosynthetic systems

In the higher organism there is strong evidence that the synthesis of the haemoprotein haemoglobin is *nor*

man Nevertheless, in the normal homeostatic state the balance sheet of pigment production and excretion by man shows an astonishing degree of efficiency, approximately 460 μ moles of haemoglobin being synthesised per day with a "wastage" of only about 24 μ moles of porphyrin precursors and of less than 0 2 μ moles of porphyrins in the urine (33) Similarly the efficiency of the enzymic system which directs porphobilinogen to uroporphyrinogen III rather than the I series isomer has been calculated (21) to be of the order of 99 9 %

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the hypothesis that haem is required for globin synthesis

Increasing concentrations of hemoglobin in the normoblast *nucleus* act like histone to combine with, condense and inactivate DNA (step 5). Globin synthesis can now continue only on preformed m RNA and stops when this m RNA is depleted. This DNA inhibition by hemoglobin will also prevent the formation of new ALA synthetase. However, even with cessation of the synthesis of this enzyme haem formation could continue for many hours after globin production has stopped by utilization of the preformed ALA synthetase. This would lead to an overproduction of haem and porphyrins. Since, under normal conditions this overproduction does not occur there must be an additional control mechanism to regulate the synthesis of ALA. It is postulated in step 6 that the free haem appearing after the termination of globin synthesis acts directly to inhibit ALA synthetase and prevent an overproduction of porphyrins" as is the case in *R. Spheroides* systems. This ingenious scheme provides a background against which to discuss recent observations upon experimental porphyrias.

Experimental porphyrias

The first experimental production of a porphyric state was accomplished by Stokvis (57) in 1895 by the admi*nistration of sulphonal* to rabbits and dogs. A number of other substances were subsequently shown to influence porphyrin metabolism but it is only since Schmid and Schwartz (53) demonstrated in 1952 *that* Sedormid administration caused in hepatic form of porphyria in rabbits that extensive study has been devoted to these phenomena. There is now a relatively *long and well documented* list of chemical substances which are capable of producing an experimental porphyria. Among these may be mentioned the sedormid type drugs (18), *certain* barbiturates (17) griseofulvin (9) 3,5 diethoxycarbonyl 1,4 dihydro-2,4,6-trimethyl pyridine (D.D.C.) (7, 23, 22), 2 allyloxy-3 methylbenzamide (45) hexachlorobenzene (43) and other chlorinated benzenes (51). A good deal of discussion took place as to whether the excessive amounts of porphyrins formed in the excreta were due to over production or to under utilization on account of some metabolic block. This latter view seemed supported by the fact that the different *porphyrinogenic* agents did not all produce the same pattern of porphyrin excretion however other considerations especially those of a *quantitative nature*, pointed to over production is the true explanation (18). Thus for example allylisopropylacetamide induced porphyria is not *accompanied in rabbits* by anaemia in spite of a daily loss of about one half the quantity of porphyrin which is synthesised daily for haemoglobin *production* (18). Merchante Wajchenberg and Schwartz (39) showed that liver homogenates from sedormid treated rats converted porphobilinogen *to porphyrin at the* same rate as did control homogenates

Fig 2 Hypothetical scheme for control of haemoglobin synthesis (Reproduced by permission from Granick and Levere (21))

mally subjected to controls similar to those outlined above and operative in micro organisms Thus the production of haem and of globin in erythropoietic cells seems to be regulated so that under normal conditions they arise in the required 1 1 ratio Granick and Levere (21) have constructed a hypothetical scheme embodying controls in haemoglobin synthesis which could explain many of the known facts (Fig 2) It assumes that globin genes are turned on independently of haem synthesis that because of globin synthesis the rate of haem synthesis is increased and that no globin polypeptide is completed in the absence of haem The steps in the scheme are explained as follows

Prior to the initiation of haemoglobin synthesis the operon responsible for globin synthesis is activated but kept repressed by its corresponding regulator gene as in step 1 The operon concerned with the synthesis of ALA synthetase, the limiting enzyme in the haem biosynthetic chain, is also repressed However, here it is assumed that the aporepressor produced by regulator gene 1 requires haem for effectively repressing the operon for ALA-synthetase (step 2) With the entrance of specific *effector* substances into the cell the *repressor* of the globin operon is rendered inactive (step 3), allowing globin synthesis to commence Erythropoietin or possibly metabolites from neighbouring cells are substances which might act in this manner to initiate globin synthesis The globin thus formed now utilizes any available free haem for hemoglobin formation (step 4) depriving the repressor of the ALA synthetase operon of its effectiveness and allowing the synthesis of this enzyme to proceed at a maximal rate Since the other enzymes in the haem biosynthetic chain are already present in non limiting amounts haemoglobin production is at this point limited only by the availability of substrates

To explain the 1 1 ratio of the haem to globin monomer formed by the red cells it is postulated that the globin will not come off the polysome unless it has been correctly folded around the haem Rich has suggested that when two adjacent monomers are completed they will come off the polysome together as a dimer Hammel and Bessman (25) found that haem stimulates globin synthesis when added to a preparation of hemolyzed avian erythrocytes which consisted mostly of nuclei This supports

sumably haem formation proceeded readily on addition of ALA to cultures at a time when addition of succinate and glycine was without effect. By passing ALA synthetase and providing intracellular haem in this way was found to stimulate globin synthesis and haemoglobin production. The level at which this effect is exerted appears to be at the ribosomal synthesis of globin since it was sensitive to puromycin but not to actinomycin D. Wilt (65) has been led to similar conclusions.

Concomitant metabolic disturbances caused by porphyrinogenic drugs

As early as 1955 Schwartz (53) observed a disturbance of lipid metabolism in experimental porphyria and an *increased rate of fatty acid synthesis* by the livers of such rats was demonstrated by Labbe, Hanawa and Lottsfeldt (29) and raised serum cholesterol, total lipids and phospholipids by Taddeini, Nordstrom and Watson (58). Reviewing records of 51 cases of hepatic porphyria the latter authors (58) found hypercholesterolaemia to be of common although inconstant occurrence in acute intermittent and mixed porphyria. Increased ascorbic acid excretion in the rat with experimental porphyria has also been observed by Ginsburg and Dowdle (16) and De Matteis (6). Such metabolic disturbances are by *no* means characteristic of the experimental porphyric state, in fact they are frequent accompaniments of the administration of a variety of different drugs. They do, however, suggest a stimulation of enzyme synthesis in the liver and the increases in ascorbic acid excretion and in hepatic UDPG dehydrogenase activity which follow intoxication with chloretone or barbital are both preventable by ethionine. In the case of the porphyrinogenic drug AIA, De Matteis (6) found that the increased excretion of ascorbic acid was not significantly affected by administration of glucose, actinomycin D or ethionine whereas each of these substances inhibits the porphyria in rats. It would appear that there is no close connection between porphyria on the one hand and increased lipid and ascorbic acid synthesis on the other.

Human Acute Intermittent Porphyria

In any attempt to relate findings from drug induced experimental porphyrias in animals to the diseases occurring in man, one is met at the outset with the difficulty that very little is known about the enzyme levels in the tissues of human porphyrics. This is largely due to the difficulty of obtaining sufficient liver and bone marrow material for adequate quantitative studies. Only in one case of acute intermittent porphyria is there definite evidence of a raised level (between 7 and 14 fold) of hepatic ALA synthetase (59). Other enzymes of the porphyrin biosynthetic pathway were present in normal amount with the exception of ALA dehydratase which was possibly slightly increased. These *important* findings are strongly suggestive of an

On the other hand Gibson (15) noted that the liver and kidney, but not bone marrow or spleen, of sedormid intoxicated rabbits showed a marked rise in ALA-dehydratase activity Granick and Urata (22) then made the important observation that the concentration of ALA synthetase in guinea pig liver was increased forty-fold above its normally low level by dosing the animals with DDC Other enzymes of the biosynthetic chain were not appreciably affected, they are normally fairly active in liver These findings indicated that the rate of hepatic porphyrin synthesis is limited primarily by the activity of ALA synthetase

From the discussion set out in the earlier part of this paper it will be clear that an increase in activity of a tissue enzyme could result from either activation of temporarily inhibited enzyme or from a *de novo* synthesis of the enzyme by the tissue cells That the porphyrinogenic drug did in fact induce new synthesis of enzyme followed from the finding (19) that the same porphyrin increase could be observed by adding AIA to chick embryo liver cells growing in tissue culture but that the effect was blocked by also adding inhibitors of protein synthesis like actinomycin D By this elegant technique two new substances chloretone and aminopyrine were added along with AIA, DDC Griseofulvin, hexachlorobenzene, sulphonal and Dial to the list of porphyrinogenic agents (19) A still more extensive list which includes many well known therapeutic agents, is given by Granick (20), they are all contra indicated for subjects known to have porphyria

It is of interest that in this tissue culture system the addition of haem to the medium did not repress the induction of porphyrin synthesis by AIA (19), whereas it will be remembered that it does repress ALA synthetase formation in *R Spheroides* (32) The explanation of the difference may, however, lie in a possible impermeability of the liver cells to haem

Assuming that haem is necessary for repression in this system also, then reference to Fig 2 will indicate that the de repression of the operator gene responsible for ALA-synthetase production could be brought about by either (1) combination of the inducing (de repressing) agent with haem itself or (2) combination with the apo repressor so as to block its union with haem In either case, the net result would be prevention of formation of the apo repressor/haem complex At the present time it is impossible to distinguish between these alternative possibilities but the way is clearly open for chemical investigation Despite wide differences in overall chemical structure among the known porphyrinogenic drugs work within particular series has already succeeded in defining the features essential for activity within such a series (18 37) In later experiments Levere and Granick (34) have used the chick blastoderm to study haem and globin synthesis *in vitro* ALA synthetase was again found to be the limiting factor for haem synthesis by these erythropoietic cells since porhyrin and pre

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protein nature and may be an enzyme which deviates succinic acid into pathways other than that of porphyrin biosynthesis

It will thus be clear that although the concept of a genetic abnormality resulting in a failure of repression of ALA synthetase in acute intermittent porphyria is attractive there remain many problems to be solved before this hypothesis can be fully accepted In this connection a profitable line of study might be the excessive catalase production observed in a *R Spheroides* mutant by *Clayton and Smith* (5) This organism would appear to present another example of a genetic defect resulting in failure to control the synthesis of a particular enzyme catalase could comprise from 5 to 25 per cent of the total dry weight of the mutant

Addendum

A recent report (28 a) states that induction of a succinyl CoA isoenzyme occurs prior to that of ALA synthetase in livers of mice receiving DDC Tricarboxylic acid cycle turnover is unchanged but succinate and succinyl CoA pools increase There may be a connection between these important findings and that of Gajdos Török (14)

References

1 Batlle A M del C Benson A & Rimington C Biochem J 97 731 1965
2 Brenner S Brit Med Bull 21 244 1965
3 Brown E G Biochem J 70 313 1958
4 Burnham, B F & Lascelles, J Biochem J 87 462 1963
5 Clayton, R K & Smith C Biochem Biophys Res Comm 3 143 1960
6 De Matteis F Biochim Biophys Acta 82 641 1964
7 De Matteis F & Prior B E Biochem J 83 1 1962
8 De Matteis F Prior B E & Rimington C Nature 191 363 1961
9 De Matteis F & Rimington, C Brit J Derm 75 91 1963
10 Eriksen L Porphyrin Biosynthesis and Metabolism Ciba Foundation Symposium p 185 1955
11 Faber M & Falbe Hansen I Nature 184 1043 1959
12 Falk J E Porra R J, Brown A Moss F & Larminie H E Nature 184 1217 1959
13 Fox H M Proc Roy Soc B 143 203 1955
14 Gajdos A & Gajdos Torok, M Bull Soc. Chim Biol 47 349 1965
15 Gibson K D Porphyrin Biosynthesis and Metabolism Ciba Foundation Symposium p 27 1955
16 Ginsburg A D & Dowdle E B S.A J Lab Clin Med 9 206 1963
17 Goldberg A Biochem J 57 55 1954
18 Goldberg A & Rimington, C Proc Roy Soc. B 143 257 1955
19 Granick S J biol Chem 238 PC 2247 1963
20 Granick S J Am Med. Assoc 190 475 1964
21 Granick S & Levere, R. D Progress in Haematology 4 1 1964
22 Granick S & Urata C J biol. Chem 238 821 1963
23 Haeger Aronsen B Acta Pharmacol Toxicol 18 165 1961
24 Hamilton T H Proc Nat Acad Sci Wash 51 83 1964
25 Hammel C L & Bessman S P Fed Proc 23 317 1964
26 Heady R E Jacobs N J & Deibel, R H Nature 203 1285 1964
27 Jacob F & Monod J J Molec. Biol 3 318, 1961
28 Jacob F & Monod J Cytodifferentiation and macromolecular synthesis Ed M Lock., Acad. Press N.Y., 1963 p 30

induction or de-repression of ALA synthetase but the mechanism by which it could be brought about is not known. Acute intermittent porphyria is a dominantly inherited disorder and Tschudy *et al* (59) point out that any defect of a regulator gene, that is a constitutive regulator mutation, would be expected to behave as a recessive. A constitutive operator mutation in acute intermittent porphyria is discussed by Watson *et al* (61) for congenital erythropoietic porphyria could explain the genetic and biochemical findings but one would have to accept the over-production of porphyrin precursors as responsible for the neurological symptoms, which is contrary to experience. Alternatively there may be a single genetic defect which lies outside of the haem biosynthetic pathway but which leads secondarily to induction of ALA synthetase.

End product repression might be defective if, for example, haem were involved in repression of hepatic ALA synthetase as it appears to be in micro-organisms, but was not produced at the normal level owing to a partial block in the biosynthetic pathway. There is, however no evidence for such a block. Another possibility which should be considered is that in acute intermittent porphyria there is a defect in an apo-repressor molecule which normally only becomes effective as a repressor of the operator gene for ALA synthetase when it is combined with a product such as haem. Inability so to combine would deprive the system of control by this mechanism.

Support for the idea that there is an induction of ALA synthetase in acute intermittent porphyria (59) is afforded by the effect of glucose. Rose, Hellman and Tschudy (52) first observed that the degree of experimental porphyria produced in animals by administration of a porphyrinogenic drug was influenced by the diet, a high carbohydrate or protein diet largely suppressing the porphyric disturbance. Later this observation was extended to the human patient with acute intermittent porphyria (63). The induction of hepatic ALA synthetase by AIA was found to be inhibited by glucose (60).

The ability of carbohydrate to inhibit the induction of certain enzymes has been observed in the past, mainly in bacteria. The phenomenon is called the 'glucose effect' or 'catabolite repression'. It has been suggested that glucose-sensitive enzymes are capable of converting their substrates into metabolites which the cell can also obtain independently and more readily from glucose whereas this is not so in the case of glucose-insensitive enzymes. The difficulty about applying this concept to the induction of ALA synthetase is that no route to porphyrins from glucose has been demonstrated. In this connection the demonstration of the biosynthesis by *R. Spheroides* of an inhibitor of porphyrinogenesis which is accelerated by ATP may prove to be of much significance. According to Gajdos and Gajdos-Török (14) the inhibitor is of

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Some recent advances in the problem of erythropoietic porphyria

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It is indeed a genuine pleasure and a great honor to contribute to a Festschrift for Jan Waldenström s 60th birthday This is all the more true in view of my long admiration of his work and character His contributions to our knowledge of porphyria in general are well known It therefore seemed appropriate to discuss some new findings and concepts related to erythropoietic porphyria

The concept that porphyria congenita Gunther is basically related to erythropoiesis and might best be designated as porphyria erythropoietica in contradistinction to the much larger group of hepatic porphyrias was first presented in 1951 (1) It was based on the observation that the bone marrow in congenital porphyria contains large numbers of fluorescing normoblasts (2 3) and the circulating erythrocytes invariably contain excessive uro and coproporphyrin in contrast to hepatic porphyria in which the liver rather than the developing red cells is rich in porphyrins or porphyrin precursors There is also the interesting fact that when excessive hemolysis was eliminated by means of splenectomy in congenital porphyria the amount of uroporphyrin in the circulating blood and excreta declined sharply These findings have been confirmed both in additional cases of the human disease and in the highly similar bovine form (4) Our group in collaboration with the Veterinary School of Medicine has had available for several years a small herd of these animals including both those homozygous for the trait with pink teeth and marked photosensitivity and heterozygotes without any discoverable abnormalities either clinical or biochemical The erythropoietic nature of the bovine disease is similarly apparent in respect to the many fluorescing normoblasts in the bone marrow and the striking effect of the stimulus provided by bleeding This is followed promptly by an outspoken increase of the circulating erythrocyte porphyrins especially uroporphyrin and concomitantly the urinary uro

28a Labbe R F, Kurumada, T & Onisawa J Biochim Biophys Acta *111* 403 1965
29 Labbe, R F Hanawa J & Lottsfeldt F I Arch Biochem Biophys *92* 373 1961
30 Lascelles J Biochem J *62* 78 1956
31 Lascelles J Biochem J *66* 65, 1957
32 Lascelles J J Gen Microbiol *23* 487 1960
33 Lascelles, J Tetrapyrrole biosynthesis and its regulation W A Benjamin Inc New York & Amsterdam 1964
34 Levere R D & Granick, S Proc Nat Acad Sci *54* 134 1965
35 Ludwig G D McNair Scott R & Chaykin, L Fed Proc *24* Part 1 223 1965
36 Marks G S Ann Reports Chem Soc *59* 385 1963
37 Marks G S Hunter E G Terner U K & Schneck D Biochem Pharmacol *14* 1077 1965
38 Massey V Biochim Biophys Acta *38* 447 1960
39 Merchante, A Wajchenberg B L & Schwartz, S Proc Soc Exp Biol Med 95 221 1957
40 Minakami S Yoneyama Y & Yoshikawa H Biochim Biophys Acta *28* 447 1958
41 Moss F Austr J Exp Biol Med Sci *34* 395 1956
42 Nishida G & Labbe R F Biochim Biophys Acta *31* 519 1959
43 Ockner R K & Schmid R Nature *189* 499 1961
44 Porra R J & Falk J E Biochem Biophys Res Comm 5 179 1961
45 Prato V Mazza U & Ramello A Lancet *i* 1276 1964
46 Rabinovitz M & Olson M E Nature *181* 1665 1958
47 Rimington C Ann Report Chem Soc *51* 311 1955
48 Rimington C Brit Med J *ii* 189 1956
49 Rimington C Rev Pure and Applied Chem *8* 129 1958
50 Rimington C Brit Med Bull *15* 19 1959
51 Rimington C & Ziegler G Biochem. Pharmacol. *12* 1387 1963
52 Rose J A Hellman E S & Tschudy D P Metabolism *10* 514 1961
53 Schmid R & Schwartz S Proc Soc. Exp Biol Med *81* 685 1952
54 Schulman M P & Richert D A J biol Chem 226 181 1957
55 Schwartz S Fed Proc *14* 717 1955
56 Shemin D & Kumin S J biol Chem *198* 827 1952
57 Stokvis B J Z klin Med *28* 1 1895
58 Taddeini, L Nordstrom K L & Watson C J Metabolism *13* 691 1964
59 Tschudy D P Perlroth M G Marver H S Collins A Hunter G & Rechcigl M Proc Nat Acad Sci *53* 841 1965
60 Tschudy D P Welland F H Collins A & Hunter G Metabolism *13* 396 1964
61 Watson C J Runge W Taddeini L Bossenmaier I & Cardinal R Proc Nat Acad Sci *52* 478 1964
62 Waxman H S & Rabinovitz M Biochem Biophys Res Comm *19* 538 1965
63 Welland F H Hellman E S Gaddis E M Collins A Hunter G W & Tschudy D P Metabolism *13* 232 1964
64 Whipple G H Haemoglobin plasma protein and cell protein Their production and interchange C C Thomas Springfield 1948
65 Wilt F H J Molec Biol *12* 331 1965
66 Wintrobe M M Harvey Lectures 45 87 1950
67 Yčas M & Drabkin D I J biol Chem 224 921 1957
68 Yčas M & Starr T J J Bact *65* 83 1953

From the Dept of Medicine University of Minnesota Hospital Minneapolis Minn Aided by grants from the U S P H S

Some recent advances in the problem of erythropoietic porphyria

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It is indeed a genuine pleasure and a great honor to contribute to a Festschrift for Jan Waldenström's 60th birthday This is all the more true in view of my long admiration of his work and character His contributions to our knowledge of porphyria in general are well known It therefore seemed appropriate to discuss some new findings and concepts related to erythropoietic porphyria

The concept that porphyria congenita Gunther is basically related to erythropoiesis and might best be designated as porphyria erythropoietica in contradistinction to the much larger group of hepatic porphyrias was first presented in 1951 (1) It was based on the observation that the bone marrow in congenital porphyria contains large numbers of fluorescing normoblasts (2 3) and the circulating erythrocytes invariably contain excessive uro and coproporphyrin in contrast to hepatic porphyria in which the liver rather than the developing red cells is rich in porphyrins or porphyrin precursors There is also the interesting fact that when excessive hemolysis was eliminated by means of splenectomy in congenital porphyria the amount of uroporphyrin in the circulating blood and excreta declined sharply These findings have been confirmed both in additional cases of the human disease and in the highly similar bovine form (4) Our group in collaboration with the Veterinary School of Medicine has had available for several years a small herd of these animals including both those homozygous for the trait with pink teeth and marked photosensitivity and heterozygotes without any discoverable abnormalities either clinical or biochemical The erythropoietic nature of the bovine disease is similarly apparent in respect to the many fluorescing normoblasts in the bone marrow and the striking effect of the stimulus provided by bleeding This is followed promptly by an outspoken increase of the circulating erythrocyte porphyrins especially uroporphyrin and concomitantly the urinary uro

28a Labbe R F Kurumada T & Onisawa J Biochim Biophys Acta *111* 403 1965

29 Labbe R F Hanawa J & Lottsfeldt F I Arch Biochem Biophys *92* 373 1961

30 Lascelles J Biochem J *62* 78 1956

31 Lascelles J Biochem J *66* 65 1957

32 Lascelles J J Gen Microbiol 23 487 1960

33 Lascelles J Tetrapyrrole biosynthesis and its regulation W A Benjamin Inc New York & Amsterdam 1964

34 Levere R D & Granick S Proc Nat Acad Sci 54 134 1965

35 Ludwig G D McNair Scott R & Chaykin L Fed Proc 24 Part 1 223 1965

36 Marks G S Ann Reports Chem Soc *59* 385 1963

37 Marks G S Hunter L G Terner U K & Schneck D Biochem Pharmacol *14* 1077 1965

38 Massey V Biochim Biophys Acta *38* 447 1960

39 Merchante A Wajchenberg B L & Schwartz, S Proc Soc Exp Biol Med *95* 221 1957

40 Minakami S Yoneyama Y & Yoshikawa H Biochim Biophys Acta *28* 447 1958

41 Moss F Austr J Exp Biol Med Sci *34* 395 1956

42 Nishida G & Labbe R F Biochim Biophys Acta *31* 519 1959

43 Ockner R K & Schmid R Nature *189* 499 1961

44 Porra R J & Falk J E Biochem Biophys Res Comm *5* 179 1961

45 Prato V Mazza U & Ramello A Lancet i 1276 1964

46 Rabinovitz M & Olson M E Nature *181* 1665 1958

47 Rimington C Ann Report Chem Soc *51* 311 1955

48 Rimington C Brit Med J *ii* 189 1956

49 Rimington C Rev Pure and Applied Chem *8* 129, 1958

50 Rimington C Brit Med Bull *15* 19 1959

51 Rimington C & Ziegler G Biochem Pharmacol *12* 1387 1963

52 Rose J A Hellman E S & Tschudy D P Metabolism *10* 514 1961

53 Schmid R & Schwartz S Proc Soc Exp Biol Med *81* 685 1952

54 Schulman, M P & Richert D A J biol Chem *226* 181 1957

55 Schwartz S Fed Proc. *14* 717 1955

56 Shemin D & Kumin S J biol Chem *198* 827 1952

57 Stokvis B J Z Klin Med *28* 1 1895

58 Taddeini L Nordstrom K L & Watson C J Metabolism *13* 691 1964

59 Tschudy D P Perlroth M G Marver H S Collins A Hunter G & Rechcigl M Proc Nat Acad Sci *53* 841 1965

60 Tschudy D P Welland F H Collins A & Hunter G Metabolism *13* 396 1964

61 Watson C J Runge W Taddeini L Bossenmaier I & Cardinal R Proc Nat Acad Sci *52* 478 1964

62 Waxman H S & Rabinovitz M Biochem Biophys Res Comm *19* 538 1965

63 Welland F H Hellman E S Gaddis E M Collins A Hunter G W & Tschudy D P Metabolism *13* 232 1964

64 Whipple G H Haemoglobin plasma protein and cell protein Their production and interchange C C Thomas Springfield 1948

65 Wilt F H J Molec Biol *12* 331 1965

66 Wintrobe M M Harvey Lectures *45* 87 1950

67 Yčas M & Drabkin D L J biol Chem *224* 921 1957

68 Yčas M & Starr T J J Bact *65* 83 1953

2) by differential centrifugation Uroporphyrin rich cells in the bovine porphyric are more fragile to hypotonic saline (4) and like the reticulocytes are in the lesser density fraction We do not have fractional osmotic fragility data for human porphyric cells but there is strong reason to believe that they would behave in a manner opposite to that of the bovine cells In a separate study with Johnson and Schwartz (9) it has been shown that young bovine cells normal as well as porphyric are relatively more fragile while those of the human and dog are in the more resistant fraction in accord with previous observations (10)

In terms of the distinction of porphyrocytes or uroporphyrin rich cells no significant difference was found in the proportion of type 1 in the new uroporphyrin formed on incubation of PBG in hemolysates representing the more and less fragile fractions despite the fact that there was a striking difference in the native uroporphyrin content per gram of hemoglobin in these fractions A much more extensive comparison has been made of hemolysates from lesser and greater density fractions as with this method it is possible to note the degree of correlation with the percentage of reticulocytes This work is to be described elsewhere and it will suffice to note here that the differences have not been consistent nor of sufficient magnitude to suggest the presence of two distinct cell types in other words porphyrocytes representing the genetic abnormality vs normal erythrocytes (11)

B *The origin of the free porphyrins of excreta, bone and other tissues*

It has been pointed out in the past that one cannot explain the very great excess of free porphyrin in the excreta on the basis of destruction of circulating red cells even if one assumes a marked shortening of the red cell life span Illustrative data are presented in Table I

Table I *Erythropoietic porphyria Uro and coproporphyrin of excreta vs erythrocyte porphyrins*

Case M M ♀ 25
ca 200 mg urinary + fecal porphyrin d
Total circulating erythrocyte porphyrin/d ca 10 mg based on a 24h r.b c life span
Cr^{51} T 1/2 15.5 d
Thus only 0.5—0.7 mg/d calculated for 20—30 d life span

On the basis of the circulating red cell mass and the erythrocyte porphyrin concentration it may be calculated that a complete turnover every few minutes would be required to begin to account for the large amounts of uro and coproporphyrin excreted without considering storage in bone and other tissues For a time we considered the possibility that there was excretion from the porphyrin rich normoblasts in the bone marrow and in the broad sense this seems essential I believe now however that this is a passive excretion related to extrusion of the porphyrin rich nuclei Fluorescence microscopy makes it fully evident that nuclei contain much more porphyrin than the cytoplasm and the fluorescence spectrum is entirely that of uroporphyrin Fig 1 shows the fluo

porphyrin increases. As will be emphasized in the following, there is also a remarkable increase in the percentage of fluorescing normoblasts in the bone marrow.

A. *Are the normoblasts uni- or bimodal in respect to the genetic error?*

The fluorescing normoblasts in the bone marrow, both human and bovine, often exhibit nuclear inclusions which contain heme (2, 3). Whether this is actually in the form of hemoglobin has not yet been determined. The nuclei also contain a large amount of uroporphyrin, obviously much more than in the cytoplasm of the normoblast. At the time of the first recognition of these peculiarities, the possibility was suggested that there are two varieties of normoblasts in this disease, one representing the genetic error which might be termed a "porphyroblast", the other normal (2, 3). Recent histologic studies (5, 6) appear to support a bimodal concept of this type; however, we have obtained important evidence more in favor of a single modality.

1. In a study with W. Runge (7, 8) it was found that when bovine porphyrics are bled repeatedly over a period of a week or ten days sufficient to produce moderate anemia and stimulus to the bone marrow, the percentage of fluorescing normoblasts increased in striking fashion from a baseline value of 40—50 to a range in the various experiments of 82—92 %. Comparable bleeding in normal animals is attended by only a very slight increase from a resting value of 1—2 % to 2—4 %, also the rapid disappearance of fluorescence in these normal cells on exposure to UV light indicates that the porphyrin is mainly proto rather than uroporphyrin as in the porphyric cows. This finding is at least very difficult to reconcile with a bimodal concept, unless one were to assume that only "porphyroblasts" increase in response to bleeding, in contrast to normal erythroblasts. This, however, would be contrary to the general evidence that bleeding induces a marked increase of normal erythroblasts in any and all species in which this has been studied.

2. It has been held that only genetically abnormal "porphyroblasts" give rise to the porphyrocytes of circulating blood, these being the cells which contain the uroporphyrin. Larizza (5) believes that the porphyrocytes are faulty cells of short life span, fundamentally related to the increased hemolysis which is often exhibited in these cases and may be outspoken. Since the genetic enzymatic abnormality whatever it may be (and this is discussed again in the following), must include an overproduction of type I uroporphyrin, it is reasonable to assume that if the porphyrocytes could be suitably concentrated, the hemolysate of such a concentrate would be expected to exhibit a much greater formation of uroporphyrin I than that of concentrates from cells of low uroporphyrin content from the same sample of blood. There are two ways in which separation of this type can be achieved: 1) by differential osmotic lysis

blast nuclei on extrusion must provide a very considerable proportion of the large amounts of porphyrin which are either excreted or stored There is an analogy in the hyperuricemia of states associated with increased nuclear turn over such as leukemia especially after radiation and polycythemia The uricosuria is marked and, of course there is often deposition in the skeleton even with manifest gout

C *The affinity of nuclear material for uroporphyrin*

Studies with W Runge and J Yarbro have made clear that uroporphyrin readily enters the nuclei of living cells such as those of rat or bovine liver or Ehrlich ascites tumor cells The fluorescence of the porphyrin is largely or completely masked first becoming apparent on treatment with dilute HCl This was well demonstrated in the chromosomes of Ehrlich ascites tumor cells undergoing mitosis We have recently obtained evidence that nucleohistone in contrast with either DNA or histone forms some type of complex with uroporphyrin This study is still in progress and will be described in detail elsewhere The finding that uroporphyrin enters nuclei raises the question as yet unanswered whether the normoblast nuclear uroporphyrin is formed in the cytoplasm or nucleus or both

D *The problem of detection of the hetero ygous state*

It is well known that classical erythropoietic porphyria or morbus Gunther is transmitted as an autosomal recessive trait We have been unable to detect the heterozygous condition by means of porphyrin analysis either in the human or bovine disease Known bovine heterozygotes, as determined by the breeding record, have failed to reveal differences from the normal In one family in which two siblings were homozygous with characteristic phenotype, the mother and two other siblings were entirely normal both clinically and by porphyrin analyses The father died of heart disease without having had any manifestations of porphyria Heilmeyer and co workers have reported significant increases of erythrocyte uroporphyrin in asymptomatic relatives of a human porphyric and they believe that these results have permitted detection of the heterozygous condition (15)

E *An "overproduction" disease without evidence of a lack of isomerase*

It has often been stated that the basic genetic abnormality in this disease is a lack of isomerase Nevertheless it has been known since Fischer s classical studies in the case Petry that while the urine and fecal porphyrins corresponded in configuration with aetioporphyrin I the protoporphyrin of the hemoglobin of circulating red cells corresponded with aetio series III (16) This in fact, first delineated the dualism of the porphyrins in nature subsequently shown to have much wider implications in biology If there were a deficiency of isomerase as a structural genetic error

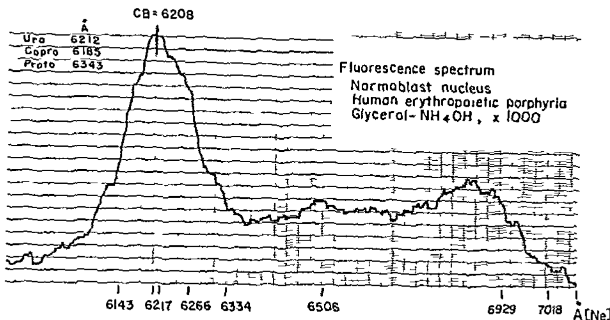


Fig. 1. Fluorescence spectrum of the nucleus of a single intact normoblast from the bone marrow biopsy of a human case of erythropoietic porphyria. Recorded by W. Runge with the microfluorospectrophotometer (12). Unstained preparation treated with *glycerol-NH_4OH 3:1*; linear magnification 1000:1. CB = center band. Ref. values from Dhére and Bois (34).

rescence spectrum of a single normoblast nucleus as recorded with the microfluorophotospectrometer, a unique apparatus which has been constructed over the past 17 years in our laboratory and is described in detail elsewhere (12). The spectral distribution curve in Fig. 1 is mainly that of uroporphyrin. This requires some explanation of the fact that approximately equal amounts of copro- and uroporphyrin are found in the excreta, the majority of the former in the feces and of the latter in the urine, in accordance with Fischer's initial discovery and nomenclature (13, 14). The most reasonable explanation is that uroporphyrin (ogen) liberated from the normoblasts is converted to coproporphyrin, either in the bone marrow or in the liver, to be excreted mainly in the bile. There is no reason to doubt that uroporphyrinogen may be converted in part to coproporphyrinogen either in the cytoplasm of normoblasts or in non-nucleated cells, especially reticulocytes. Excessive coproporphyrin in the circulating red cells, especially the lesser density fraction, is readily demonstrable. There is also the question whether uro- or coproporphyrin can be secreted by or in some manner lost to the plasma from intact erythrocytes. We have not been able to detect any loss from freshly collected bovine porphyric red cells when kept in motion in plasma, incubated over a number of hours. This, it is recognized, does not exclude the possibility that there might be secretion in vivo. All things considered, however, it seems clear that the uroporphyrin-laden normo-

the enzyme incubated in vitro with coproporphyrinogen I there was no evidence of formation of protoporphyrin It is nevertheless conceivable that the enzyme might partially lose its specificity under certain circumstances in vivo While these observations are of considerable interest the amounts of the differing mesoporphyrins and the frequency of their occurrence was too small to indicate a fundamental significance for the disease and its genetic abnormality

In respect to the concept of an isomerase deficiency it is important to emphasize that there is actually a considerable overproduction of type III porphyrin including protoporphyrin in this disease For one thing the free porphyrins of the excreta although mainly type I include a distinct proportion of type III ranging from 5 to 20 % in various determinations This represents a much larger amount than is found in the normal excreta More important however is the fact that there is generally a considerable excess of urobilinogen in the feces This has been shown 1) not to differ from the stercobilin of normal feces (20) and 2) to consist in large proportion of the early labelled fraction (20 21 22) The general significance of the early labelled bile pigment has been considered in a recent review (23) and need not be dealt with in any detail at present It is often related in considerable measure to ineffective erythropoiesis of which there is essentially no evidence in erythropoietic porphyria The bone marrow in this disease is usually hyperplastic and normoblastic and there is good reason to believe that the heightened erythropoiesis is a response to increased red cell destruction at least in part by the spleen However a marked increase in early labelling has been noted even when there was no evidence of increased hemolysis (20 22) It is evident that the extrusion of the normoblast nuclei containing heme inclusions as referred to in the foregoing provides a ready source of heme for conversion to bile pigment either in the bone marrow or after transport to the liver This may not be the only source of early labelling in this disease but it is reasonable to believe that it is a highly important one From the standpoint of the question of isomerase deficiency these findings indicate that there is actually an overproduction of heme and consequently of protoheme 9 and the corresponding bile pigment

If the amounts of type III porphyrin and urobilinogen of the excreta are compared with those of the type I porphyrins and with average normal data it is quite evident that there is a highly significant overproduction of type III as well as type I although the amount of the latter is relatively much greater than in the normal individual and this of course is basic to the phenotype of the disease Such a comparison is shown in Fig 2 in which the essential data from one of our cases of erythropoietic porphyria is contrasted with average normal data

Erythropoietic porphyria is thus to be regarded as a disease of overproduction rather than actual enzyme deficiency or block The overproduction

one would anticipate a striking diminution in heme and hemoglobin synthesis with a hypochromic anemia except if there were compensatory excessive formation of type I proto heme or if the isomerase deficiency were limited to a relatively small colony of abnormal cells Fischer could not detect evidence of the first of these possibilities showing that the protoporphyrin of Petry hemoglobin was type 9 corresponding with the aetio III series This depended on conversion of proto to mesoporphyrin Fischer commented on the fact that the mother liquor was dark, hence the possibility of a small proportion of type I isomer could not be excluded (16) My associates and I have reexamined this question in considerable detail (17) In the earlier course of our investigations a number of years ago prior to the availability of chromatographic techniques now available, the sodium salt solubility (16) and melting points of porphyrin methyl esters obtained after hydriodic acid reduction of hemin suggested the presence of a type I isomer Later, however it became clear that the hydriodic acid method yields other unidentified porphyrins in addition to mesoporphyrin In all of our more recent studies protoporphyrin has been prepared from hemoglobin powder by the method of Grinstein (18) The total protoporphyrin thus obtained has then been converted to mesoporphyrin by catalytic hydrogenation with palladium following which a sample of the entire mesoporphyrin has been subjected to paper chromatography (7 17) A sample of crystalline hemin from the case Petry was very kindly provided by Prof Alfred Treibs Technische Hochschule, Munchen After conversion to proto, thence to mesoporphyrin, only type 9 (III) was demonstrable by paper chromatography Many samples of bovine and human porphyric hemoglobins, both from circulating red cells and from those of the spleen, have thus been examined in a search for an isomeric protoporphyrin In all but three instances there was no suggestion of any porphyrin but type 9 in the meso fraction The three exceptions included the spleen from a newborn porphyric calf the spleen from a case of human porphyria and one sample of bovine porphyric red cells A distinct spot was observed on the paper chromatogram of each of these having the same R_f as mesoporphyrin 1 The human spleen yielded a sufficient amount of a differing mesoporphyrin in the 0 8 % NaOH soluble fraction to permit crystallization This comprised but a small proportion of the total mesoporphyrin most of which was in the 0 8 % NaOH insoluble fraction The crystalline methyl ester from the soluble fraction exhibited the dimorphic melting point of mesoporphyrin 1 in addition to the paper chromatographic behavior of this isomer The rare occurrence of small proportions of type 1 isomer as represented in these three exceptions is not understood Sano and Granick (19) have shown that the coproporphyrinogenase responsible for conversion of coproporphyrinogen III to protoporphyrinogen 9 (III) is highly specific for type III With a relatively pure sample of

(porphobilinogen) In acute porphyria much of the excess of ALA and PBG is *promptly excreted* by the liver cells and appears in the urine In erythropoietic porphyria an increased amount of these precursors formed in the normoblasts is at once converted to uro and coproporphyrinogen the type III moiety of which is then largely converted to proto heme while the type I porphyrins are disposed of as already discussed Excesses of PBG and ALA in the urine in erythropoietic porphyria have not been observed and since an excessive formation of these substances may be taken for granted the lack of increase in the urine is best explained on the basis of retention in the developing red cells and rapid conversion to porphyrin

F *Other forms of erythropoietic porphyria*

In my discussion of classical erythropoietic porphyria attention must also be given to the remarkably different protoporphyria first described by Magnus and Rimington (26) and which has been studied so extensively and to such good purpose in Professor Waldenstrom s clinic especially by Dr Birgitta Haeger Aronsen Reference may be made to her excellent survey *of various aspects of this curious* disease in the recent literature (27) There is little doubt that many cases have been classified as solar erythema urticaria or eczema in the past without recognition that they were suffering from porphyria partly because of the usual absence of a vesiculo bullous eruption (hydroa), but more important, because reliance was placed on urinary porphyrin analyses and because protoporphyrin is not excreted in the urine It is now fully established that the most constant feature of the disease is the remarkably heightened erythrocyte protoporphyrin concentration *Our experience* with a number of cases of this well defined form of porphyria has been described in a recent paper (28)

Heilmeyer and Clotten (29) have described what they regard as an independent form of erythropoietic porphyria i e "coproporphyria" It is highly interesting that their case was the same as that of Kosenow and Treibs (30) published 12 years earlier under the title of "Lichtüberempfindlichkeit und Porphyrinämie" They noted many fluorescing erythrocytes but were unable to identify the erythrocyte porphyrin with certainty as protoporphyrin They found however that the plasma contained a porphyrin metal complex which they stated was not that of protoporphyrin on spectroscopic grounds Nevertheless the feces contained a large amount of protoporphyrin and very little coproporphyrin and this of course *is quite in accord* with protoporphyria There was no increase of urinary porphyrin *With these observations in mind the find*ings of Heilmeyer and Clotten 12 years later are of great interest The urine was *again entirely normal* The erythrocytes contained large amounts of coproporphyrin entirely type III isomer *a moderate increase* of proto and a relatively marked increase of uro

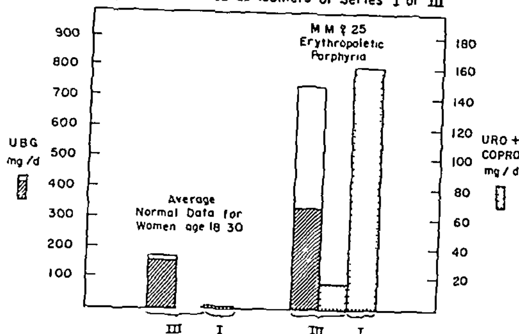


Fig 2 Amounts of porphyrin isomers *I and III* and urobilinogen 9α (III) in the excreta in erythropoietic porphyria as contrasted with average normal values for same sex and age range The upper *open areas in the* urobilinogen (UBG) columns simply indicate that a much larger proportion is of the *"early labelled"* type in erythropoietic porphyria than in the normal This was calculated in terms of total circulating hemoglobin and Cr^{51} T 1/2 (Table I)

is characterized, however, by an imbalance in which the deaminase activity is relatively much greater than if the normal ratio had been maintained Taking into account the recessive character of the disease a porphyrin over production such as this represents is satisfactorily explained as a constitutive regulator mutation with induction of δ aminolevulinic acid (ALA) synthetase, in the developing red cells Various possibilities based on such a mutation have been discussed in detail in a separate communication (7) Granick (24) has shown quite clearly that porphyrogenic chemicals act by derepression and consequent induction of ALA synthetase in liver cells and Tschudy (25) has recently demonstrated a highly significant increase of ALA synthetase in the liver of a fatal case of human acute porphyria The striking phenotypic differences between acute porphyria and erythropoietic porphyria assuming that ALA synthetase induction is common to both, might well be explained on the basis of differences between liver cells and normoblasts, both in terms of permeability to ALA and PBG and to relative rates of utilization of these compounds as a function of the concentration of enzymes in the porphyrin biosynthetic pathway beyond PBG

9 Johnson L Schwartz S and Watson C J in preparation
10 Harris J W The red cell Harvard Univ Press 1963 p 208
11 Watson C J Bossenmaier I and Cardinal R in preparation
12 Runge W Science in press
13 Fischer H Ztschr f physiol Chem 95 34 1915
14 *Fischer H* Ibid 96 148 1915
15 Heilmeyer L Clotten R Kerp L, Merker H, Parra C A and Wetzel H P Deutsch med Wchnschr 88 2449 1963
16 Fischer H and Kirrmann A Ann d Chemie 475 266 1929
17 Watson, C J Schwartz S Kay I T, Aziz M Bossenmaier I and Cardinal, P in preparation
18 Grinstein M J Biol Chem 167 515 1947
19 Sano S and Granick S J Biol Chem 236 1173 1961
20 Gray C H Muir J M H and Neuberger A Biochem J 47 542 1950
21 Grinstein M Aldrich R A Hawkinson V Lowry P and Watson C J, Blood J of Hematol 6 699 1951
22 London I M West R Shemin D and Rittenberg D J Biol Chem 184 365 1950
23 Watson C J Proc 9th Congr Europ Soc Haemat Lisbon 1963 S Karger Basel New York p 817
24 Granick S Trans N Y Acad Sc 25 53 1962
25 Tschudy D P, Perlroth M G, Marver H S Collins A Hunter G Jr and Rechcigle M Jr Proc Nat Acad Sc, 53 841 1965
26 Magnus I A, Jarrett A Prankerd T A J and Rimington C Lancet 2 448
27 Haeger Aronsen B, Am. J Med 35 450 1963
28 Peterka E S Fusaro R M, Runge W J Jaffe M. O and Watson C J J Am. Med Assn 193 120 1965
29 Heilmeyer L and Clotten R Deutsch. med Wchnschr 89 649 1964
30 Kosenow W and Treibs A, Ztschr f Kinderheilk 73 82 1953
31 Goldberg A and Rimington C. Diseases of porphyrin metabolism C C Thomas Springfield Ill. 1962 p 40
32 Watson C J, A.M.A Arch Int Med 99 323 1957
33 *Watson C J in Grundlagenforschung* in ihrer Bedeutung fur die Klinische Medizin" Internationales Symposion in der Med Univ Klinik Freiburg i Br anlasslich des 65 Geburtstages von Herrn Professor Dr Dr h c L Heilmeyer Herausgeben von Prof Dr W Keiderling F K Schattauer Verlag Stuttgart 1965
34 Dhére, Ch and Bois E., C. R Acad Sci. Paris 183 321 1926

porphyrin, in comparison with the normal. No porphyrins were demonstrable in the plasma. The feces contained a normal amount of copro- and normal to slightly increased values for 2 COOH porphyrins (This would include at the least, proto-, meso- and deuteroporphyrins). It is evident that these data, especially for the fecal porphyrins, differ considerably from those of Kosenow and Treibs 12 years earlier. The absence of any plasma porphyrin or of any increase of urinary porphyrin in association with such marked increase of erythrocyte coproporphyrin is indeed unexpected. Heilmeyer and Clotten believed this might be due to an abnormal oxidation of coproporphyrinogen in the red cells, with accumulation until the end of their life span. On the basis of Rimington's observations (31) this would explain the lack of increase of urinary coproporphyrin and the amount derived from red cell destruction would be too small in any event, to result in significant increases of urinary or fecal porphyrin. Thus at a level of 450 μg of coproporphyrin per 100 ml of red cells, a red cell volume of 2000 ml and a 120 day red cell life span it may be calculated that only 80 μg/d would be available for excretion from the destruction of mature circulating erythrocytes. This is inconsequential in relation to the normal daily excretion of coproporphyrin (32). Even at a markedly increased rate of red cell destruction the amount of coproporphyrin might easily fall within the normal range of excretion. Assuming that the hypothesis of Heilmeyer and Clotten is correct, the principal question which remains is why the patient had photosensitivity at all. With such a small amount derived from red cell destruction and no porphyrin demonstrable in the plasma, it is difficult to understand how sufficient porphyrin might accumulate in the skin to produce photocutaneous injury. Obviously of paramount importance to this question is the microfluorospectrophotometric study of skin biopsies such as Runge has carried out in protoporphyria (33).

From the foregoing it will be evident that further study of additional cases of this type is much needed.

Let me close with all good wishes to my friend Jan Waldenstrom on the occasion of his sixtieth birthday and the hope that he will have many years of health, and happiness in his work and with his family.

References

1. Watson, C. J., Lowry, P. T., Schmid, R., Hawkinson, V. E. and Schwartz, S.: Trans. Assn. Am. Phys. 64: 345, 1951.
2. Schmid, R., Schwartz, S. and Watson, C. J.: A.M.A. Arch. Int. Med. 93: 167, 1954.
3. Schmid, R., Schwartz, S. and Sundberg, D.: Blood 10: 416, 1955.
4. Watson, C. J., Perman, V., Spurrell, F., Hoyt, H. and Schwartz, S.: A.M.A. Arch. Int. Med. 103: 436, 1959.
5. Larizza, P.: Pan minerva medica 4: 31?, 1962.
6. Gross, S., Schoenberg, M. D. and Mumaw, V. R.: Blood J. of Hematol. 25: 49, 196?.
7. Watson, C. J., Runge, W., Taddeini, L., Bossenmaier, I. and Cardinal, R.: Proc. Nat. Acad. Sc. 52: 478, 1964.
8. Runge, W. and Watson, C. J.: in preparation.

9 Johnson L., Schwartz S and Watson C J in preparation
10 Harris J W The red cell Harvard Univ Press 1963 p 208
11 Watson C J Bossenmaier I and Cardinal, R in preparation
12 Runge W Science in press
13 Fischer H Ztschr f physiol Chem 95 34 1915
14 Fischer H Ibid 96 148 1915
15 Heilmeyer L Clotten R Kerp L Merker H Parra C A and Wetzel H P Deutsch med Wchnschr 88 2449 1963
16 Fischer H and Kirrmann A Ann d Chemie 475 266 1929
17 Watson C J Schwartz S Kay I T., Aziz M Bossenmaier I and Cardinal R in preparation
18 Grinstein M J Biol Chem 167 515 1947
19 Sano S and Granick S J Biol Chem 236 1173 1961
20 Gray C H Muir I M H and Neuberger A Biochem J 47 542 1950
21 Grinstein M Aldrich R A Hawkinson V Lowry P and Watson C J Blood J of Hematol 6 699 1951
22 London I M West R Shemin D and Rittenberg D J Biol Chem 184 365 1950
23 Watson C J Proc 9th Congr Europ Soc Haemat Lisbon 1963 S Karger Basel New York p 817
24 Granick S Trans N Y Acad Sc 25 53 1962
25 Tschudy D P Perlroth M G Marver H S Collins A Hunter G Jr and Rechcigle M Jr Proc Nat Acad Sc 53 841 1965
26 Magnus I A., Jarrett A Prankerd T A J and Rimington C Lancet 2 448
27 Haeger Aronsen B Am J Med 35 450 1963
28. Peterka E S Fusaro R M Runge W J Jaffe M. O and Watson C J J Am Med Assn 193 120 1965
29 Heilmeyer L and Clotten R Deutsch. med Wchnschr 89 649 1964
30 Kosenow W and Treibs A Ztschr f Kinderheilk 73 82 1953
31 Goldberg A and Rimington C Diseases of porphyrin metabolism C. C. Thomas Springfield Ill 1962 p 40
32 Watson, C J A.M.A Arch Int Med 99 323 1957
33 Watson C J in "Grundlagenforschung in ihrer Bedeutung fur die klinische Medizin" Internationales Symposion in der Med Univ klinik Freiburg i Br anlasslich des 65 Geburtstages von Herrn Professor Dr Dr h c L Heilmeyer Herausgegeben von Prof Dr W Keiderling F K Schattauer Verlag Stuttgart 1965
34 Dhéré Ch and Bois E. C R Acad Sci Paris 183 321 1926

porphyrin, in comparison with the normal No porphyrins were demonstrable in the plasma The feces contained a normal amount of copro- and normal to slightly increased values for 2-COOH porphyrins (This would include at the least, proto, meso- and deuteroporphyrins) It is evident that these data, especially for the fecal porphyrins, differ considerably from those of Kosenow and Treibs 12 years earlier The absence of any plasma porphyrin or of any increase of urinary porphyrin in association with such marked increase of erythrocyte coproporphyrin is indeed unexpected Heilmeyer and Clotten believed this might be due to an abnormal oxidation of coproporphyrinogen in the red cells, with accumulation until the end of their life span On the basis of Rimington's observations (31) this would explain the lack of increase of urinary coproporphyrin, and the amount derived from red cell destruction would be too small in any event to result in significant increases of urinary or fecal porphyrin Thus at a level of 450 μg of coproporphyrin per 100 ml of red cells, a red cell volume of 2000 ml and a 120 day red cell life span it may be calculated that only 80 $\mu g/d$ would be available for excretion from the destruction of mature circulating erythrocytes This is inconsequential in relation to the normal daily excretion of coproporphyrin (32) Even at a markedly increased rate of red cell destruction the amount of coproporphyrin might easily fall within the normal range of excretion Assuming that the hypothesis of Heilmeyer and Clotten is correct, the principal question which remains is why the patient had photosensitivity at all With such a small amount derived from red cell destruction and no porphyrin demonstrable in the plasma, it is difficult to understand how sufficient porphyrin might accumulate in the skin to produce photocutaneous injury Obviously of paramount importance to this question is the microfluorospectrophotometric study of skin biopsies such as Runge has carried out in protoporphyria (33)

From the foregoing it will be evident that further study of additional cases of this type is much needed

Let me close with all good wishes to my friend Jan Waldenstrom on the occasion of his sixtieth birthday and the hope that he will have many years of health, and happiness in his work and with his family

References

1 Watson C J Lowry P T Schmid R Hawkinson V E and Schwartz S Trans Assn Am Phys 64 345 1951
2 Schmid R Schwartz S and Watson C J A M A Arch Int Med 93 167 1954
3 Schmid R Schwartz S and Sundberg D Blood 10 416 1955
4 Watson C J Perman V Spurrell F Hoyt H and Schwartz S A M A Arch Int Med 103 436 1959
5 Larizza P Pan minerva medica 4 315 1962
6 Gross S Schoenberg M D and Mumaw V R Blood J of Hematol 25 49 1965
7 Watson C J Runge W Taddeini L Bossenmaier I and Cardinal R Proc Nat Acad Sc 52 478 1964
8 Runge W and Watson C J in preparation

domonas Spheroides in mixture II of Lascelles containing ALA as substrate (Gajdos and Gajdos Török 1963 a)

Our experiments have shown that ATP reduces the porphyrin biosynthesis probably by the enhancement of the formation of a physiological inhibitor Our observations seem also to show that this inhibitor favours the utilisation of succinate for metabolic pathways others than that of ALA synthesis (Gajdos and Gajdos Török 1963 a)

These findings have led us to study in rats the effect of administration of compounds capable of reducing the biosynthesis of ATP We have thought that if our earlier conclusions are correct these compounds would determine an excess formation of porphyrins

Three chemically highly different compounds were utilised 6 mercaptopurine orotic acid and ethionine They were administered in separate experiments to female adult rats maintained in metabolic cages on balanced diet when not otherwise stated

We summarize briefly our observations published elsewhere in details

I 6 mercaptopurine was given by gastric tube in a daily dosis of 20 or 40 mg/kg during 4 weeks (Gajdos and Gajdos Török 1963 b)

The biosynthesis of adenine nucleotides was controled in the red cells As it is shown in fig 1 a statistically significant decrease was observed in the level of ADP and ATP with an equally significant increase in the concentration of AMP

It seems therefore that intoxication with 6 mercaptopurine has lead in our experimental conditions to the lowering of the biosynthesis of ATP by partly reduction of the phosphorylation of AMP

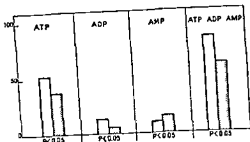


Fig 1 Levels of purine nucleotides in red blood cells (µg/100 ml) of normal rats and of rats intoxicated with 6-mercaptopurine (5 mg/rat/day) during 2 weeks
White bars mean values of 9 normal rats
Shadowed bars mean values of 4 rats intoxicated with 6-mercaptopurine

As to the porphyrin synthesis we have noted the stimulation of urinary excretion of ALA and coproporphyrin (in one experiment for instance the mean values were raised from 7 0 to 48 2 and from 2 2 to 14 0 µg/rat/day respectively without appearance of uroporphyrin in the urine The increase in the hepatic level of ALA Pbg and etherosoluble porphyrins (copro and protoporphyrin) was statistically significant

The excess formation of porphyrins caused by intoxication with 6 mercaptopurine was significantly reduced by administration of AMP or inosine at the daily dosis of 20 mg/rat

II For the study of effect of *orotic acid* on porphyrin metabolism this compound was mixed to a balanced

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Excess porhyrin formation following administration of inhibitors of the biosynthesis of ATP

By A GAJDOS (Paris)

The extremely low quantity of free porhyrins in comparison with the quantity of these pigments incorporated into hemoproteins (hemoglobin, myoglobin etc) indicates a highly effective regulatory mechanism of the biosynthesis of porphyrins This mechanism is at present poorly understood

We have recently observed, that ATP may play an important role in this point of view (Gajdos A and Gajdos-Torok M 1963 a)

In our studies on the effect of purine nucleosides and nucleotides on the synthesis of porphyrin by Rhodopseudomonas Spheroïdes incubated semianaerobically in the light in medium I of Lascelles (containing glycine and succinate as substrates) we have observed an almost complete inhibition of the synthesis by addition of ATP to the incubation mixture at the concentration of 3 m M (Table 1) Incubation semianaerobically in light during 48 hours of Rhodopseudomonas spheroides suspended in mixture I of Lascelles (5 5 mg microorganisms in 10 ml) The purine nucleosides and nucleotides were added to the incubation mixture at the concentration of 3 m M

Table 1

	Dry weight of micro organisms mg/ml	Porphyrins formed µmM/ml	Bacterio chlorophyll µmM/ml
Without addition	1 2	36 9	1 69
With adenine	1 1	29 5	1 25
With adenosine	0 9	14 6	0 84
With inosine	1 2	19,3	1 65
With AMP	1 2	7 9	1 35
With ADP	1 0	10 9	1 40
With ATP	1 1	0 5	1,35

The inhibitory effect of AMP and ADP was not only lesser but also slower than that of ATP indicating that the former nucleotides may act as precursors of ATP

We have also established that the inhibition by ATP is located to the synthesis of δ aminovulinic acid (ALA) In fact, the porphyrin synthes is was not modified when ATP was added to a suspension of Rhodopseu

Table 3

	Rats intoxicated with ethionine during 1 week	Controls
Liver ATP (µmol/100 wet w)	79±7.0 (mean of 14 rats)	168±11 (mean of 14 rats)
	$t=6.3$ $P<0.01$	
Etherosoluble porphyrins in liver (µg/100 g wet w)	17.1±2.3 (mean of 12 rats)	11±1.0 (mean of 12 rats)
	$t=2.3$ $P<0.05$	
Etherosoluble porphyrins in red blood cells (µg/100 ml)	62±7.2 (mean of 12 rats)	33±3.5 (mean of 12 rats)
	$t=3.6$ $P<0.01$	

coproporphyrin from 3 to 17.7, protoporphyrin from 21.4 to 492.3 µg/g dried feces.

We have noted a highly significant *decrease* in the hepatic level of ATP and a similarly significant *increase* in the concentration of etherosoluble porphyrins in the liver and red blood cells (*table 3*).

We have not found an increase in the activity of ALA synthetase in the liver mitochondria. Granick (1963) has recently observed that compounds capable of inducing enhanced porphyrin synthesis determine the de novo formation of the enzyme in the mitochondria of hepatic cells. Our negative findings after administration of ethionine may be explained by the inhibition of protein synthesis by this toxic agent, as it was shown by Farber et al. (1964) and Weber et al. (1964).

Administration of AMP or inosine or ATP did not reduce in our experiments the excess of porphyrin formation caused by intoxication with ethionine. This inefficacy of adenosine nucleosides and nucleotides — the only one we have observed in the numerous experimental porphyrias — may be explained by the inhibitory effect of ethionine on protein synthesis. In fact our experimental findings have indicated that the effect of ATP on excess porphyrin biosynthesis consists in the stimulation of the formation of a physiological inhibitor, which our observations have shown to be proteinic in nature.

In every case the determinism of an experimental porphyria in rats by administration of three differents compounds — 6-mercaptopurine, orotic acid and ethionine — which have the common character of being inhibitors of ATP biosynthesis furnishes further arguments in favour of the regulatory role of this nucleotide in the biosynthesis of porphyrins.

References

Euler von L. H., Rubin H. J. and Handschuhmacher R. E. (1963). Journ. Biol. Chem. 238, 2464.

Farber E., Shull K. H., Villa Trevino S., Lombardi B., and Thomas M. (1964). Nature 203, 34.

Gajdos A., and Gajdos-Torok, M. (1963 a). Bull. Soc. Chim. Biol. 45, 857.

Table 2

	Rats intoxicated with orotic acid during 3 weeks	Controls
Liver ATP (µmol/100 g wet w)	60 ± 8.7 (mean of 10 rats)	164 ± 11.0 (mean of 10 rats)
	t = 4.6	$P < 0.001$
Hepatic level of etherosoluble porphyrins (µg/100 g wet w)	16.2 ± 0.9 (mean of 8 rats)	3.4 ± 1.4 (mean of 6 rats)
	t = 4.0	$P < 0.01$
Etherosol porphyrins in red blood cells (µg/100 ml)	69.0 ± 3.5 (mean of 8 rats)	30.6 ± 1.95 (mean of 10 rats)
	t = 5.6	$P < 0.001$
Etherosol porphyrins in pooled bone marrow (µg/10^{10} red blood cells)	38 (mean of 8 rats)	23 (mean of 8 rats)
ALA synthetase activity in liver mitochondria (µmol ALA formed/mg protein of mitochondria)	10.8 ± 3.5 (mean of 4 rats)	0 to 4.5 (10 rats)

diet at the rate of 1 % (Gajdos and Gajdos Török 1965 b)

During the 3 weeks of administration of orotic acid, the mean value of fecal etherosoluble porphyrins raised from 50 to 313 µg/g dry feces On the contrary, the urinary excretion of porphyrins and porphyrin precursors was not significantly modified

At the end of the third week, the animals were killed In the liver, a highly significant decrease in the level of ATP was observed confirming the findings of Von Euler et al (1963) The concentration of etherosoluble porphyrins was raised in the liver red blood cells and bone marrow On the other hand an important increase in the activity of ALA synthetase was noted in the liver mitochondria

As in the intoxication with 6 mercaptopurine, the excess porphyrin formation caused by orotic acid was significantly reduced by administration of AMP or inosine at the daily dosis of 20 mg per rat

III *Ethionine* was shown by Stekol et al (1960) and others to strongly lower the hepatic level of ATP Gibson et al (1962) reported that this amino acid stimulates porphyrin synthesis in Rhodopseudomonas Spheroïdes Thus, the utilisation of this compound in our experiments was particularly interesting

We report here only the result of one of our experiments (Palma Carlos et al 1965) where a daily dosis of 50 mg ethionine per rat was administered by gastric tube during one week to rats maintained on relatively low protein diet (12 % casein)

The renal excretion of ALA and coproporphyrin was raised at the end of the intoxication respectively from 5.7 to 19.5 and from 0.5 to 3.6 µg/Rat/day An even more clearcut increase was noted in the level of fecal porphyrins

Table 3

	Rats intoxicated with ethionine during 1 week	Controls
Liver ATP (µmol/100 wet w)	79 ± 7 0 (mean of 14 rats)	168 ± 11 (mean of 14 rats)
	t = 6.3 P < 0 01	
Etherosoluble porphyrins in liver (µg/100 g wet w)	17 1 ± 2.3 (mean of 12 rats)	11 ± 1 0 (mean of 12 rats)
	t = 2.3 P < 0 05	
Etherosoluble porphyrins in red blood cells (µg/100 ml)	62 ± 7 2 (mean of 12 rats)	33 ± 3 5 (mean of 12 rats)
	t = 3 6 P < 0 01	

coproporphyrin from 3 to 17 7 protoporphyrin from 21 4 to 492 3 µg/g dried feces

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Administration of AMP or inosine or ATP did not reduce in our experiments the excess of porphyrin formation caused by intoxication with ethionine This inefficiency of adenosine nucleosides and nucleotides — the only one we have observed in the numerous experimental porphyrias — may be explained by the inhibitory effect of ethionine on protein synthesis In fact our experimental findings have indicated that the effect of ATP on excess porphyrin biosynthesis consists in the stimulation of the formation of a physiological inhibitor which our observations have shown to be proteinic in nature

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References

Euler von L. H Rubin H J and Handschumacher R E (1963) Journ Biol Chem 238 2464

Farber E Shull K H Villa Trevino S., Lombardi, B., and Thomas H (1964) Nature 203 34

Gajdos, A., and Gajdos Torok, M (1963 a) Bull. Soc Chim Biol. 45 857

Gajdos A, and Gajdos Török, M (1963 b) C R Soc Biol *157* 1965

Gajdos, A and Gajdos Török M (1965 a) Nature *207* 640

Gajdos A, and Gajdos Török, M (1965 b) Nature in press

Gibson K P, Neuberger A and Tait, G H (1962) Biochem Journ *83* 550

Granick S (1963) Biochem Journ *238* 2247

Palma Carlos A, Palma Carlos L Gajdos Török M and Gajdos, A (1965) Nature in press

Stekol J A, Mody, U Bedrak E Keller and Perry J (1960) Feder Proceed *19* 37

Weber et al cited by Turnes, M, and Kand Reid E (1964) Nature *203* 1174

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The Relationship between the Neurological and Biochemical Lesions in *Acute Intermittent Porphyria*

By C H Gray

Until nearly a decade after the publication of Waldenström's monograph on porphyria (31) the nervous system lesions on the one hand and constipation with abdominal colic on the other were accepted as due to a toxic action of porphyrins on the nervous system and the gut respectively This view received some support from early pharmacological studies using impure preparations of porphyrins although Waldenström himself mentioned the possible importance of vasospasm in causing the abdominal abnormalities Lowry Schmid Hawkinson Schwartz & Watson (19) suspected that porphobilinogen or a closely related substance might be responsible for the clinical manifestations However Goldberg Paton & Thompson (14) showed that purified porphyrins and porphobilinogen are pharmacologically inactive and produced evidence against the possible existence of a circulating vaso constrictor substance in acute porphyria Later Jarrett Rimington & Willoughby (18) showed that δ aminolaevulic acid the biosynthetic precursor of porphobilinogen excreted in large amounts in the urine in acute porphyria, was similarly without pharmacological action Goldberg (13) suggested all the clinical features of acute porphyria could be explained on a neurological basis the gastro intestinal features being attributed to lesions of the preganglionic motor fibres that innervate the viscera Degenerative changes were found in the nuclei of nerve cells in the spinal cord and medulla which give rise to these fibres

The earlier work on the changes in the nervous system in acute porphyria have been admirably summarised by Hierons (17) who confirmed earlier observations of Denny Brown & Sciarra (7) and others in demonstrating abnormalities in the anterior horn cells but disagreed with their concept that the nerve lesions were secondary to vascular abnormalities Hierons considered that the vascular lesions occurring in the brain were probably secondary to the severe hypertension which is characteristic of the disease and that the mental and neurological

disturbances were due to a direct, possibly reversible, metabolic effect of the disease resulting in some affection and loss of neurones in the brain and cerebellum and spinal cord Goldberg (13), however, had emphasised the importance of primary demyelination

The controversy concerning the histopathological changes has been the subject of a recent annotation in the Lancet (2) The peripheral neuropathies may be due to a primary abnormality of the axon or to primary damage to the myelin In the former there is a "dying back" process in the peripheral nerves and the demyelination is secondary to it, in the latter the demyelination, which is segmental and patchy, is in the Schwann cells and nerve sheaths and results in the axon degeneration Erbsloh (9), Mason, Courville & Ziskind (20) and Simpson (25) all found evidence suggesting that the damage to the peripheral nerves in acute porphyria affected primarily the axons On the other hand, Denny-Brown & Sciarra (7) had observed segmental demyelination in the peripheral nerves of two patients while Gibson & Goldberg (12), in a study of peripheral and autonomic nerves, found demyelination occurring earlier to a greater degree than the damage to the axons More recently Cavanagh & Mellick (4) have examined the muscles, peripheral nerves, spinal cord and root ganglia in four subjects with porphyria They correlated the clinical features of their cases with the extent of the lesions and with the distribution of the motor and sensory nerves in the affected muscles Regardless of the period of paralysis, there is gross denervation of muscle, the degeneration in the nerves being of the dying back form characteristic of primary axonal disease Unlike the neuropathy of organo phosphate poisoning in which the distal muscles with the longest nerve fibres are affected earliest and most extensively, the dying-back in acute porphyria occurs irrespective of the distance of the muscle from the spinal cord The severity of the weakness will therefore depend upon the size of motor unit affected In addition to the motor neurones which are particularly affected, sensory fibres in the nerves and sensory roots were involved although there was no clinical indication of sensory disturbances Heirons (17), and Goldberg (13) had previously shown that sensory nerves may be affected Cavanagh & Mellick (4) emphasised our ignorance of the rate of ascent of the lesions and point out that the histopathological changes may only be obvious in those subjects surviving longest They suggest that in those subjects in whom recovery occurred the distances over which regeneration of the nerve takes place must be relatively short

There is now little doubt that all the clinical features of acute intermittent porphyria are directly due to changes in the nervous system Goldberg (13) postulated the formation of a substance X of which porphobilinogen was a precursor essential for nutrition of myelin of the nervous system He postulated that in acute porphyria there was a metabolic block in the for

mation of A which leads to an increased excretion of porphobilinogen and also causes demyelination A primary defect in the motor neurones with a lesser abnormality in the sensory neurones could not be due to such a disturbance of myelin metabolism but might follow from an abnormality of acetylation and succinylation De Matteis and Rimington (6) proposed a deficiency of acetyl choline synthesis due to an increased competition for glycine due to succinyl coenzyme A The condensing enzyme responsible for the condensation of succinyl coenzyme A with glycine to form ALA can also bring about the condensation of acetyl coenzyme A with glycine to form amino acetone If for some reason the rate of production of acetyl coenzyme A were limited the resulting deficiency of acetylcholine could interfere with transmission of the nerve impulse and larger quantities of succinyl coenzyme A might react with glycine to form ALA Such a theory would suggest that amino acetone is a normal metabolite in the human subject and that amino acetone would be absent from the urine of patients with acute porphyria However Tschudy Welland Collins & Hunter (28) Drum m & Hager Aronsen (8) found no differences between the aminoacetone excretion of patients with acute porphyria and that of normal subjects

Rimington (23) has pointed out that many of the features of acute porphyria could be attributed to defective synthesis of acetylcholine because of interference with the acetylating mechanism, a deficiency of coenzyme A or of acetyl coenzyme A from pyruvate of DPN or of available energy from ATP The effect of barbiturates on porphyria could be due to the effect of this drug in uncoupling oxidative phosphorylation which would decrease the availability of ATP for the synthesis of acetyl coenzyme A This might provide a basis for the apparent benefit of adenosine monophosphate or inosine phosphate treatment of acute porphyria [Gajdos & Gajdos Török (11)] However Urata & Granick (29) have shown that two distinct enzymes are responsible for the synthesis of aminoacetone and ALA respectively

There has been much speculation as to whether the excessive excretion of ALA and PBG was due to an over production or to under utilisation of ALA There might be either a block in the catabolism of one of the precursors of ALA via an alternative pathway not leading to ALA or PBG or to a block in the metabolism of ALA itself The site of such a block has not been identified Richards & Scott (22) showed that it was unlikely that any of the major pathways of glycine metabolism were blocked and they attempted to assess the magnitude of the Shemin pathway by which ALA is deaminated to give α oxoglutaraldehyde which would then be degraded to succinic acid and a one carbon atom fragment They investigated the ability of the body to convert glycine to serine a process requiring such a one carbon atom fragment but showed that in only 3 of 6 patients with

disturbances were due to a direct, possibly reversible, metabolic effect of the disease resulting in some affection and loss of neurones in the brain and cerebellum and spinal cord Goldberg (13), however, had emphasised the importance of primary demyelination

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acute porphyria may not be caused directly by the formation of ALA and PBG a metabolic event perhaps in the nervous system or elsewhere might be responsible both for the nervous system lesion and for the excretion of the metabolites in excess

Until the precise metabolic abnormality is known and the precipitating features identified the absence of correlation between ALA and PBG excretion and clinical symptoms and the great variation in the clinical picture in the disease cannot be understood In some patients the motor nervous system is affected and they are paralysed in others the autonomic nervous system is mainly affected and there is abdominal pain The central nervous system abnormalities develop dramatically reaching their maximum within a few hours The patient may then be left with residual abnormality even though there has been recovery from the metabolic disturbance responsible Such acute changes followed by return to the metabolic state characteristic of remission may account for the inconsistent results obtained by Richards & Scott (22) in investigating the conversion of glycine to serine and also account for the observations by Smith & Taylor (26) that the α oxoglutarate concentration in the blood of patients with acute porphyria was increased in only 4 out of 5 subjects and the failure of others to confirm their findings There is urgent need to re investigate many of these problems during the acute stage of the disease and not at a time when a spontaneous recovery and remission is probably taking place

The similarity between the neurological disturbances in lead poisoning and those characteristic of acute porphyria and the increased excretion of ALA and coproporphyrin III in the former condition suggests that a more detailed study of liver and brain metabolism in both would be worthwhile particularly because experimental porphyria in animals is never accompanied by lesions of the nervous system whereas these are characteristic in experimental lead poisoning

Carbohydrate has been shown to inhibit the induction of ALA synthetase of AIA in experimental animals [Tschudy Welland Collins & Hunter (28)] and also to reduce the excretion of ALA and PBG by patients with acute intermittent porphyria [Welland Hellman Gaddis Collins Hunter & Tschudy (33)] These workers discuss their findings in relation to the catabolite repression of enzyme induction in which catabolites which are formed rapidly from glucose accumulate in the cell and repress the formation of enzymes whose activity would augment the already large intracellular pools of these compounds They found it difficult to invoke the concept of catabolic repression to explain the effect of glucose on ALA synthetase The important role of glucose in brain metabolism would make it highly desirable that this repressive effect of carbohydrate should receive careful re investigation Perhaps the elegant investigations now being carried out by Gaitonde (10) Balazs (3) and Vrba

acute porphyria was the conversion of glycine to serine abnormally low These findings require confirmation and further investigation

The primary metabolic lesion in acute porphyria has still not been identified despite the demonstration that in the experimental porphyrias induced by allylisopropylacetamide (AIA) or dicarbethoxydihydrocollidine (DCC) there is an induction of a greatly increased synthesis of the enzyme ALA synthetase [Granick & Urata (15)] They have postulated that these porphyrinogenic agents act in antagonising a repressor gene controlling the formation of ALA More recently Perlroth Tschudy Marver, Collins, Hunter & Rechcigl (21) have shown that in acute intermittent porphyria in human subjects the hepatic ALA synthetase is greatly increased They agree that this provides some support for the view of Watson Runge Taddeini Bossenmaier & Cardinal (32) that there is a constitutive operator mutation for ALA synthetase and that the neurological manifestations are a direct consequence of the over production of ALA and PBG They point out however that the condition might be due to a defective structural gene lying outside the porphyrin biosynthetic pathway and that the induction of ALA synthetase is a secondary phenomenon the primary defective structural gene would then lead to an enzyme deficiency and predispose the patient to the clinical features of the disease

Many workers have failed to appreciate the great capacity for recovery shown by patients with severe nervous system lesions in acute porphyria the histological findings described by numerous authors may account not for the acute lesions but for the residual abnormalities of the central nervous system which characterises many patients between relapses This reversibility of the major neurological disturbances associated with an acute attack provides important support for Hierons' concept that the vascular lesions reported by Denny-Brown & Sciarra (7) were probably secondary to hypertension and that the mental and nervous system disturbances were due to direct and probably metabolic effects of the disease comparable to the effects of mescaline and similar drugs Ackner, Cooper, Gray Kelly & Nicholson (1) emphasised the absence of correlation between PBG and ALA excretion and the clinical symptoms and reiterated the view that the excretion of the two metabolites and the nervous system changes are separate manifestations of a metabolic event or series of events However they admitted that it was possible that a severe acute attack with neurological manifestations might be associated for a short period with a sudden burst of excessive excretion of the two porphyrin precursors The association of neurological symptoms and signs in acute porphyria with the excretion of ALA and PBG in the urine may be analogous to the relationship between diabetic coma and ketosis Coma may be accompanied but not necessarily caused by the production of ketonic acids and similarly the neurological lesions of

18 Jarrett A Rimington C & Willoughby D A (1956) Lancet i 125

19 Lowry P T., Schmid R Hawkinson V E., Schwartz S & Watson C J (1950) Bull Univ Minn Hosp 22 97

20 Mason R. Courville C & Ziskand E (1933) Medicine (Baltimore) 12 355

21 Perlroth M Tschudy D., Marver H Collins A Hunter G & Rechcigl M (1965) J clin Invest 44 1085

22 Richards F F & Scott J J (1961) Clin Sci 20 387

23 Rimington C (1961) Symp on the Normal & Pathological Metabolism of Porphyrins Turin 12th June 1961 Panminerva Medica

24 Schwartz S (1955) Fed Proc 14 717

25 Simpson J A (1962) in Progress in Electromyography" Ed Pinelli P Buchthal F & Thiebaut F., Elsevier Amsterdam

26 Smith M J H & Taylor K W (1954) Unpublished observations

27 Taddeini L., Nordstrom K I & Watson, C. J (1964) Metabolism 13 691

28 Tschudy D P Welland F H., Collins A & Hunter G W (1963) Lancet, 2 660

29 Urata G & Granick S (1963) J biol. Chem 238 811

30 Vrba R (1962) Nature 195 663

31 Waldenstrom J (1937) Acta med scandinav (Supp) 82

32 Watson C J Runge W., Taddeini, L., Bossenmaier I & Cardinal R (1964) Proc Nat Acad Sci 52 478

33 Welland F H Hellman E S Gaddis E. M., Collins A Hunter G W., Tschudy D P (1964) Metabolism 13 232

(30) at the Medical Research Council Neuropsychiatrical Research Unit in London, could be extended to investigate the effect of any of the porphobilinogenuric drugs on brain metabolism They have shown that although in abnormal animals a part of [U-^{14}C] glucose is metabolised directly via the tricarboxylic cycle to carbon dioxide, a substantial part is retained in the brain as amino acids, proteins and lipids There was a particularly high incorporation into glutamate which *not only undergoes transamination to* α oxoglutarate but is oxidised via gamma aminobutyric acid (GABA) and succinic aldehyde They calculate that in the steady state the turnover rate of glucose through GABA was about 10 % of turnover through the tricarboxylic acid pathway An investigation of the metabolism of glutamate and GABA in acute porphyria might well be rewarding

Liver lipid phosphorus and total lipid have shown to be increased in sedormid porphyria [Schwartz (24)] as is fatty acid synthesis in the liver of AIA treated rats More recently Taddeini, Nordstrom & Watson (27) have found a marked elevation of cholesterol total lipids and phospholipids after AIA and DCC in rabbits Gray & Waterfield (16) have also shown that two porphyrinogenic agents profoundly disturbed the incorporation of ^{14}C from [U ^{14}C] glucose into protein and glycogen However De Matteis (5) has shown that some of the metabolic changes which accompany the disturbance of ALA and PBG synthesis can also occur after the administration of related drugs not producing experimental porphobilinogenuria He therefore, believes that the association of these changes with increased porphyria formation should be regarded as coincidental There is clearly urgent need to investigate further the metabolic pathways either directly or indirectly related to ALA synthesis in both brain and liver and perhaps other tissues of the body

References

1 Ackner B G C Cooper J E Gray C H Kelly M & Nicholson D C (1961) Lancet *i* 1256

2 Annotation Lancet (1965) 2 1336

3 Balazs R (1965) Biochem J *95* 497

4 Cavanagh J B & Mellick R S (1965) J Neurol Neurosurg Psychiat *28* 320

5 De Matteis F (1966) Biochem J *98* 23c

6 De Matteis F & Rimington C (1962) Lancet *i* 1332

7 Denny Brown D & Sciarra D (1945) Brain *68* 1

8 Druyan R & Haeger Aronsen B (1964) Scandinav J Clin & Lab Invest *16* 498

9 Erbsloh W (1903) Dtsch *7* Nerven heilk *23* 197

10 Gaitonde M K Dahl D R & Elliott K A (1965) Biochem J *94* 345

11 Gajdos A & Gajdos Torok M (1961) Lancet *2* 175

12 Gibson J B & Goldberg A (1956) J Path Bact *71* 495

13 Goldberg A (1959) Quart J Med *28* 183

14 Goldberg A Paton W D & Thompson J W (1954) Brit J Pharmacol 9 91

15 Granick S & Urata G (1963) J biol Chem *238* 821

16 Gray C H & Waterfield M D (1965) Proc II Int Symposium on Porphyrins St Vincent, Italy

17 Hierons R (1957) Brain *80* 176

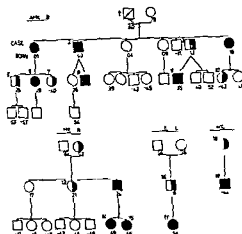


Fig. 1. Four families with protoporphyria erythropoietica. Squares represent males and circles females; solid symbols manifest cases, semi-solid symbols latent cases. Hollow symbols unaffected members. Diagonal across symbol indicates that the member has not been examined.

the opportunity to make in our first case (7—8). We showed that the disease is genetically determined and that its heredity is probably dominant. This assumption was later corroborated by others and by further personal investigations. It is known for instance that a pair of American identical twins were affected with the disease (25).

Material

Our Swedish material consists of 24 cases belonging to 5 families (fig. 1)[1]. One family, B, of them can be traced back to Norrland, the northern part of Sweden, from where also most cases of acute intermittent porphyria have been reported (25—30). Genetical studies in which the ancestors were followed back to the beginning of the 19th century, however, failed to reveal any blood relationship between this family and families with acute porphyria.

[1] One family ... ares 0—24 will be published later by Dr. H. Hansson and Prof. ... Th..., Uppsala, Sweden.

Of our 24 cases half occurred in males and half in females. Fifteen cases were clinically manifest while the remaining 9 were asymptomatic but the patients had an increased concentration of protoporphyrin in the red blood cells (7) or in the faeces (2). The 9 asymptomatic cases were classified as latent.

Case reports

Family B

Case 1. S.J. 64-year-old woman. Housewife. Since childhood she has suffered from discomfort, almost nausea, on exposure to sunshine. No other general symptoms. The skin becomes red but not tanned. Grey-blue eyes, previously fair hair, now grey.

Case 2. C.B. 69-year-old man. Unskilled labourer. Since 20 years of age he has had itching of the skin on exposure to sunshine. He reported that he also may develop pepper-corn-sized blisters which heal without scars. The cutaneous changes only appear on the hands, never on other areas of the skin, not even after long exposure to sunshine. The patient tans readily. Blue eyes, brown hair.

Case 4. L.W. 4 year-old woman. Housewife. Has never liked to sunbathe. Cannot tolerate sunshine but is unable clearly to define the symptoms. On exposure to the sun the skin never tans but only turns temporarily red without other symptoms. On one occasion in the spring of 1963 when the patient was in the mountains the face became red and swollen. No vesicles or pealing of the skin. She recovered after one week. Grey-brown eyes, hair previously fair, now grey.

From the Departments of Internal Medicine and Dermatology, *General Hospital, Malmö, Sweden*

Erythropoietic Protoporphyria

A study of known cases in Sweden

By Birgitta Haeger Aronsen and Gösta Krook

Protoporphyria erythropoietica (P.E.) became the focus of extensive interest after its rediscovery in 1961 by Rimington and coworkers, who gave the disease its name (17). It had been described as early as 1953 in Germany (12), but had for some reason apparently fallen into oblivion.

Clinically, the disease is characterised by an abnormal sensitivity to light, which usually makes its first appearance within the first two years of life. A few minutes' exposure to sunshine is enough to cause intense painful itching in the areas exposed. This is followed by erythema, oedema and usually crust formation, especially on the nose, cheeks and ears. Occasionally eczematous changes occur. The skin becomes coarse, and affected children then look old for their age. This is thought to depend on the presence of large amounts of hyaline material deposited around the blood vessels of the upper corium (20). The nails are often opaque and have no lunulae. Vesicles, hyperpigmentation and hirsutism sometimes develop, though rarely.

The laboratory findings are dominated by a markedly increased concentration of protoporphyrin in the red blood cells, the plasma and the faeces. The faecal coproporphyrin content may be slightly increased, while the urinary porphyrins and their precursors, δ-aminolaevulinic acid and porphobilinogen, occur in normal amounts.

Microscopic examination in ultraviolet light of erythrocyte smears shows abundant bright red fluorescing cells.

Some 50 cases of P.E. have been reported in countries outside Scandinavia. Most cases have been seen in U.S.A. (9, 10, 21, 23—26), but several also in Great Britain (3, 11, 17), Germany (12—15) and France (1—2). One case has been described in South Africa (27). The frequency of the disease does not vary with sex. In the first German and British cases no family studies were made, which we had

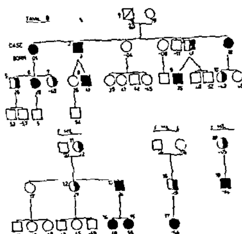


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Of our 24 cases half occurred in males and half in females. Fifteen cases were clinically manifest, while the remaining 9 were asymptomatic but the patients had an increased concentration of protoporphyrin in the red blood cells (7) or in the faeces (2). The 9 asymptomatic cases were classified as latent.

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[1] One family (cases 20—24) will be published later by Dr H. Hansson and Prof. N. Thyresson, Upsala, Sweden.

Case 6 B Ö 37 year old woman Clerk Her skin becomes red on exposure to sunshine Never tans Never itching oedema or other skin symptoms No discomfort on exposure to the sun Grey blue eyes, brown hair

Case 8 R B 24 year old man Shop assistant Abnormally sensitive to sunshine already at one month of age After about five minutes exposure in summer or about 30 minutes in winter the skin begins to itch with a sensation of burning and pricking in the exposed areas Sometimes he also has chills which continue for days and disturb his sleep The itching is followed some hours later by oedema and erythema After severe exposure pinheadsized vesicles occasionally occur When after 1—3 days the oedema has abated fissures form which heal leaving behind small scars The skin on the back of the hands is sometimes yellow white or blue yellow Sometimes the skin lesions are accompanied by watering of the eyes Some hours after exposure to sunshine he may pass 2—3 loose dark stools and sometimes also big volumes of urine which however looks normal The symptoms are not confined to any particular season of the year but are most marked between February and August Grey blue eyes dark hair

Case 9 B B 30 year old man Engineer Abnormally sensitive to sunshine since 6 years of age Exposed areas of the skin feel irritated burn and are tender No itching Sometimes erythema oedema and petechiae occur On one occasion only when he was in the mountains the skin blistered No crusts or scars develop No hypertrichosis or increased brittleness of the skin Normal pigmentation after exposure to sunshine Exposure has never caused any systemic symptoms such as chills or nausea Grey blue eyes brown hair

Family R

Cases 13 14 and 15 correspond to cases 5 12 and 13 in a previous publication (8)

Family L

Case 17 C L 10 year old girl Since 4 years of age the patient has had itching pain and sensation of burning in the skin in areas exposed to sunshine These symptoms are often accompanied by chills She occasionally has oedema but never erythema Small pale milia sometimes appears but no blisters Crusts form and peal after a few days When after 1—2 months they have fallen off they leave behind small irregular scars Grey blue eyes fair hair

Family V

Case 19 L V 21 year old man Electrician Since 3 years of age he has had itching and sensation of burning in sun exposed areas of the skin Erythema and diffuse oedema as well as chills sometimes occur and only on a few occasions has the skin blistered but never was there left behind any scars Does not tan readily not even in summer Grey blue eyes very fair hair

At the examination all 8 cases reported appeared physically and mentally normaly developed The dental status was also normal Neither the liver nor the spleen was palpable In none of them the skin was brittle Case 2 is hyperpigmented all the year round The patient in case 8 was moderately pigmented over the metacarpophalangeal joints especially II—III bilaterally He had several small scars in the forehead on the tip of the nose and on the cheek The nails of the second and third fingers on either hand had no lunulae In case 9 the patient had numerous lentigenes on the arms and back but otherwise showed nothing remarkable Case 17 showed small irregular depressions on the condyles of the second and third fingers on either hand and dirty brown pigmentation of the backs of the hands and lichenification of several of the knuckles Scaling of skin on the tip of the nose giving follicular hyperkeratosis

scabs and a few scars Case 19 also showed lichenification of several knuckles but no other skin lesions All the nails were thick and had no lunulae

Three of our patients (nos 8 9 19) reported that the more often they are exposed to sunshine the quicker the symptoms appear (a loading up?) In skin tests (with Kromayer s lamp equipped with monochromatic filter Zeiss 508953) on one of our patients (no 19) itching appeared as well as a sensation of burning in areas previously exposed to sunshine already after 10 minutes but not until after three times this interval in areas not previously exposed

Four patients (nos 2 8 9 19) reported that dirt and sweat on the skin can accelerate and accentuate the symptoms One (no 2) of them reported that he has symptoms only on the hands (dirt?) even when he also exposes other areas of the body

It is remarkable that 3 of the patients (nos 1 4 6) had a hitherto undescribed relatively mild form of P E where the only symptoms were reddening of the skin sometimes intense without subsequent tanning nausea and general feeling of discomfort on exposure to sunshine

Four patients (nos 8 13 17 19) were often troubled by chills after exposure to sunshine a symptom we have not found mentioned in other case reports of P E

Another symptom not reported is the frequent passage of loose dark stools which was noted in one of our cases (no 8)

Table I Concentration of protoporphyrin in red blood cells (PP/RBC), coproporphyrin in faeces (CP/F) and protoporphyrin in faeces (PP/F) in 11 manifest (M) and 8 latent (L) cases of protoporphyria erythropoietica

Case			Time of analyses	PP/RBC μg/100 ml	CP/F μg/g dry weight	PP/F μg/g dry weight
1	S J	M	65 02	51	15	35
2	G B	M	65 02	57	7	6
3	S B	L	65 02	58	9	25
4	L W	M	65 02	53	13	41
5	H J	L	65 02	11	12	78
6	B Ö	M	65 02	56	21	102
7	G J	L	65 02	23	20	117
8	R B	M	65 02	1944	18	347
9	B B	M	65 04	593	9	136
			05	830	8	154
10	K W	L	65 02	43	9	72
11	E R	L	62 01	50	0	0
12	M Ö	L	62 01	40	0	7
13	L R	M	62 01	839	5	65
14	K R	M	62 01	211	0	11
15	E R	M	62 01	763	2	58
16	I L	L	63 05	59	4	15
17	C I	M	63 05	384	—	—
			09	625	4	23
18	S N	L	65 08	64	6	24
19	L N	M	64 04	332	15	145
			65 04	408	7	51
			08	633	6	56
Normal upper limit m+2 S D				≤25	≤6	≤35

Results

The results of the most relevant analyses protoporphyrin in red blood cells (PP/RBC) coproporphyrin in faeces (CP/F) and protoporphyrin in faeces (PP/F) in our series are given in table I All the analyses were performed by the methods described previously (1 5 6)

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An observation arguing against a decreased consumption of PP in the synthesis of haem in these patients is the largely normal Hb, serum iron and TIBC values. The sideroblasts were not increased in marrow smears. Finally the disappearance and appearance rate of iron in patients hitherto studied have been normal.

The other possibility, increased synthesis, has been tested by Porter (22) in bone marrow incubation tests. He showed that the immature *red blood* cells in these patients have an overproduction of protoporphyrin.

Contrary to what one might expect the increased concentration of PP in plasma is not due to leakage from the PP loaded red blood cells but at least partly to an overproduction of this porphyrin in the liver (11, 24).

Can the clinical symptoms be explained by the markedly increased concentration of PP in the red blood cells and plasma? Porphyrins have long been known to be photo sensitizers (28) and in other types of porphyria where these compounds meet sunshine hypersensitivity to light occurs. On examination of cases with P.E. Magnus (16–18) found the sensitivity of the skin to be greatest on irradiation with wavelengths around 400 mμ corresponding to the absorption maximum of the porphyrins. Magnus believed that the hypersensitivity of the skin in P.E. is due to the effect of the light on the PP laden red blood cells in the capillaries (18). If this were correct one should however expect a corresponding skin reaction in lead poisoning where the concentration of PP in the red blood cells sometimes reaches *the same level* as in *P.E. But* no such reaction is seen. A more plausible explanation of the hypersensitivity to light would therefore *be the* increased concentration of PP in the plasma which occurs *in P.E. patients* but not in leadworkers.

Therapy

Various ointments have been tried in the prophylaxis of P.E. but none have so far proved satisfactory. We ourselves have used red veterinary petrolatum cream with 10 % *p*-amino benzoic acid and Contralum® (Hermal Chemie, Hamburg), all without success.

Since antimalarial drugs have proved to have light protective effect in case 17 we tried chloroquine (Nivaquine® May & Baker Ltd.) 50 mg daily by mouth during the summer of 1961. The preparation was without any effect. In the summer of 1963 we changed to Triquin® (Winthrop) with a good effect. As this preparation contains not only chloroquine but also oxychloroquine and quinacrine we thought the latter component to be *responsible for the favorable effect* since it absorbs radiation particularly at the maximum of porphyrins, i.e. about 400 mμ which chloroquine and oxychloroquine do not (19). In the spring of 1964 when the patient again had severe lesions she was therefore treated with Quinacrine® (May & Baker Ltd.) alone in a dose of 50 mg every other day for 1 month. Treatment

No determinations were made of the plasma protoporphyrin. In all the cases the excretion of δ aminolaevulinic acid, porphobilinogen, uroporphyrin and coproporphyrin in the urine was normal.

As to the other laboratory studies, the haemoglobin concentration and the red and white blood cell count were determined in 7 of the patients and 4 (nos 8, 9, 13, 19) were found to have a moderate hypo- or normochromic anaemia with a minimum Hb of 12.3 g/100 ml (no 8). The number of red and white blood cells was normal in all of the patients studied. The E.S.R. was likewise normal in all 5 patients examined in this respect. The serum iron was normal in 11 patients examined while the TIBC was slightly increased in two (nos 8, 10). The serum haptoglobin and bilirubin were determined in 12 patients and were found to be normal in all. The serum electrolytes and the GOT were normal in all of the 5 patients studied in this respect. The serum GPT was measured in 12 and 2 of them (nos 8, 11) showed slightly increased values of 44 and 33 units (NV ⩽ 30 U) while the others showed normal values. The bromsulphalein retention test was found to be normal in 2 patients studied. Serum protein electrophoresis was done in 8 cases. In 4 of them the pattern was normal while 2 showed a slightly increased concentration of γ globulin (nos 1, 7) 2 a slight increase of α_2 globulin (nos 2, 7) and finally 1 had shown a moderately decreased concentration of β_1 globulin (no 4). The changes thus showed no uniform tendency.

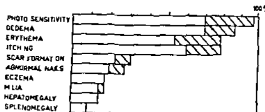


Fig. 2. Frequency of clinical symptoms in 36 manifest cases of protoporphyria erythropoietica (1—3, 10—15, 17, 21, 23—27) including 11 personal cases.

Many of our patients were examined especially for haemolysis of the red blood cells (reticulocyte count, serum haptoglobin and bilirubin) in association with exposure to sunshine, but no haemolysis occured. This is contrary to what was reported by Harris (10) and by Harber and coworkers (9) after *in vitro* studies of blood specimens from patients with P.E., when they found a most striking haemolysis after 320—450 mμ radiation.

The frequency of different clinical symptoms are given in fig. 2.

Discussion

Why then do the patients with P.E. have such a high concentration of protoporphyrin in the red cells, plasma and faeces? This porphyrin represents a normal intermediary stage in the haembiosynthesis. Once iron is incorporated in its core the synthesis is complete. An increased concentration of free PP in the red blood cells may occur either owing to a decreased consumption or increased production of this metabolite.

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had the desired effect and the patient could again tolerate sunshine But 10 days after withdrawal she had a relapse, which was, however, soon controlled on resumption of treatment Of 5 other patients (nos 8, 9, 13, 15, 19) treated with Quinacrine the drug produced the desired effect in only one (No 13)

Inosine, which Gajdos (1, 2) found to have a good effect on the symptoms of P E, was tried in one of our cases (no 9) in a dose of 4 mg/kg bodyweight (288 mg) a day, for 12 days, but without success The treatment was, however, given for a much shorter period than that recommended by Gajdos

Summing up, so far no really efficacious treatment of the symptoms of P E is available

Summary

A survey is given of all known cases of protoporphyria erythropoietica in Sweden Of the 24 cases hitherto diagnosed 15 are clinically manifest and 9 latent The patients belong to five families and we have been able to confirm the previous assumption that the disease is inherited and that the gene is dominant The clinical symptoms and biochemical abnormalities of the disease are described and the aetiology of the condition is discussed

Acknowledgement

Supported by grant from the Swedish Medical Research Council

References

1 Gajdos A Canet J Combrisson A & Gajdos Török M Nouv Rev Franç d Hematol *4* 575 1964
2 Gajdos A Gajdos Torok M Mantz J M & Schirardin H Presse Med *73* 119 1965
3 Gray C H Kulczycka A Nicholson D C Magnus I A & Rimington, C Clin Sci *26* 7, 1964
4 Haeger B Lancet *2* 606 1958
5 Haeger Aronsen B Scandinav J Clin & Lab Invest *12* (supp 47) 45 1960
6 Haeger Aronsen B Scandinav J Clin & Lab Invest *14* 397 1962
7 Haeger Aronsen B Lancet *1* 1073 1962
8 Haeger Aronsen B Am J Med *35* 450 1963
9 Harber L C Fleischer A S & Baer R L JAMA *189* 191 1964
10 Harris H Arch Derm *91* 85 1965
11 Holti G Magnus I A & Rimington C Brit J Derm *75* 225 1963
12 Kosenow W & Treibs A Ztschr Kinderh *73* 82 1953
13 Kosenow W Med Klin *49* 1099 1954
14 Langhof H Zschr Haut Geschl Krkh *28* 353 1960
15 Langhof H Muller H & Rietschel L Arch klin exp Derm *212* 506 1961
16 Magnus I A In Progress in Photobiology Proceedings of the Third International Congress on Photobiology Finsen Mem Congr p 144 Amsterdam 1961 Elsevier Publ Co
17 Magnus I A Jarrett A Prankerd T A J & Rimington C Lancet *2* 448 1961
18 Magnus I A In Les Maladies du Metabolisme des Porphyrines (Sec Colloq Internat de Biol de Saclay) p 55 Paris 1962 Presses Universitaires de France
19 Mc Chesney E W Nachod F C & Tainter M L J Invest Dermatol *29* 97 1957
20 Peterka E S Fusaro R M & Goltz R W Arch Dermatol *92* 357 1965
21 Porter F S & Lowe B A Blood *22* 521 1963
22 Porter F S Blood *22* 532 1963

23 Redeker A G Levan N & Berke M Arch Derm 86 569 1962
24 Redeker A G Bronow R S & Sterling R E South Afr J Lab Clin Med 9 235 1963
25 Redeker A G & Bronow R S Arch Derm 89 104 1964
26 Redeker A G & Bryan H G Lancet 1 1449 1964
27 Sweeney G D Dowdle E. B., Saunders S J & Eales L South Afr J Lab Clin Med 9 247 1963
28 Urbach E & Bloch J Wien klin. Wchschr 47 527 1934
29 Waldenstrom J Acta Med Scand. 92 (supp 82) 139 1937
30 Waldenstrom J Acta genet. 6 122 1956

had the desired effect and the patient could again tolerate sunshine But 10 days after withdrawal she had a relapse, which was, however, soon controlled on resumption of treatment Of 5 other patients (nos 8 9, 13, 15, 19) treated with Quinacrine the drug produced the desired effect in only one (No 13)

Inosine which Gajdos (1, 2) found to have a good effect on the symptoms of P E, was tried in one of our cases (no 9) in a dose of 4 mg/kg bodyweight (288 mg) a day, for 12 days, but without success The treatment was, however, given for a much shorter period than that recommended by Gajdos

Summing up so far no really efficacious treatment of the symptoms of P E is available

Summary

A survey is given of all known cases of protoporphyria erythropoietica in Sweden Of the 24 cases hitherto diagnosed, 15 are clinically manifest and 9 latent The patients belong to five families and we have been able to confirm the previous assumption that the disease is inherited and that the gene is dominant The clinical symptoms and biochemical abnormalities of the disease are described and the aetiology of the condition is discussed

Acknowledgement

Supported by grant from the Swedish Medical Research Council

References

1 Gajdos A Canet J Combrisson A & Gajdos Török M Nouv Rev Franç d Hematol 4 575 1964

2 Gajdos A Gajdos Torok M Mantz I M & Schirardin H Presse Med 73 119 1965

3 Gray C H Kulczycka A Nicholson D C Magnus I A & Rimington C Clin Sci 26 7 1964

4 Haeger B Lancet 2 606 1958

5 Haeger Aronsen B Scandinav J Clin & Lab Invest 12 (supp 47) 45 1960

6 Haeger Aronsen B Scandinav J Clin & Lab Invest 14 397 1962

7 Haeger Aronsen B Lancet 1 1073 1962

8 Haeger Aronsen B Am. J Med 35 450 1963

9 Harber L C Fleischer A S & Baer R L JAMA 189 191 1964

10 Harris H Arch Derm 91 85 1965

11 Holti G Magnus I A & Rimington C Brit J Derm 75 225 1963

12 Kosenow W & Treibs A Ztschr kin derh 73 82 1953

13 Kosenow W Med klin 49 1099 1954

14 Langhof H Zschr Haut Geschl Krkh 28 353 1960

15 Langhof H Müller H & Rietschel L Arch klin exp Derm 212 506 1961

16 Magnus I A In Progress in Photobiology Proceedings of the Third International Congress on Photobiology Finsen Mem Congr p 144 Amsterdam 1961 Elsevier Publ Co

17 Magnus I A Jarrett A Prankerd T A J & Rimington C Lancet 2 448 1961

18 Magnus I A In Les Maladies du Métabolisme des Porphyrines (Sec Colloq Internat de Biol de Saclay) p 55 Paris 1962 Presses Universitaires de France

19 Mc Chesney E W Nachod F C & Tainter M L J Invest Dermatol 29 97 1957

20 Peterka E S Fusaro R M & Goltz R W Arch Dermatol 92 357 1965

21 Porter F S & Lowe B A Blood 2? 521 1963

22 Porter F S Blood 22 532 1963

examination if they had a hang over as this generally meant that they would be suspected of being lead poisoned

Since 1961 the control of the workers at this factory has been performed by the author Starting at the beginning of 1963 determination of the urinary output of delta aminolevulic acid (ALA) was made in conjunction with the semi quantitative determination of coproporphyrins Since 1964 coproporphyrins have not been determined and determination of ALA has been made routinely as suggested by Haeger Aronsen (4) Determinations of ALA give a good estimate of metabolically active lead in the organism (Cramer & Selander 5)

In the present investigation a comparison is made between the living habits of lead poisoned workers and those of workers with the same degree of lead exposure without symptoms of poisoning to determine the influence of the factors mentioned above

Laboratory methods

ALA was d tern ned as I cr bed by Mauzerall a d Granick C n ll e n od f cat on of Haeg r Aro se 4 I re ous compar ons betwe ALA expres e l as g 100 ml ur ne a l ll e a ou t f ALA excrete l per gra n I cr a e Cra ér & Selander lave h w tl at for routine purposes t s not ece ary t r late tl e ou put t creat n ne I tl e w k e le l t is al o de trat d l at alues fr fresl ly led san pl s take lur g tl e day are as nfor at e as I t r al s t ade n 24 h ur pec e s A ALA c trat n f 1 a mg 100 m l ur ne was a pt le l a a pper l g n eally per tle r al l t Wo kers sl w l gher alue were cla f ed as be g lea l po e l

Clinical material

Twenty s x workers at the Tudor Storage Battery Works show g the prev ously de scr bed s g s of lead po son ng dur ng 1964 were used The workers were employed n the follow ng work sl ops of the plant (Table I) The ALA values for the two groups are g en n table II

Table I *Workshops for lead poisoned and non poisoned workers*

Ball m ll and paste m x ng	10
Format on	2
F ll ng	4
Iast ng	6
Lead recovery and cast ng	4

For every lead po soned worker a worker *w thout s ns of lead po son ng but w th the* sa e t me of employment and tl e same degree of lead exposure was exam ned In total 26 pa rs of workers were exam ned. The oral exam nat on as made by the author us ng a standard zed quest onna re The inter v ews were made w th one worker at a t m e and n str ct conf dence Tl e subjects co operated w ll ngly and nobody refused to take part n the exam nat on

Results

The results are given in table II—V There was no correlation between leisure time spent indoors and the use of tobacco in the workshop on the one hand and the frequency of lead poisoning on the other

The difference in distribution of lead poisoning between those who ate one hot meal per day and those who ate two meals or more is at the border line of statistical significance The difference between those consuming 75 cl or more of strong liquor per month and those with a lower alcohol consumption seems to be well established however

From Medical Service I, Sahlgrenska sjukhuset, University of Goteborg Göteborg Sweden and AB Tudor Nol Sweden

Predisposing Factors for Lead Poisoning

By Kim Cramer

The invention of semi quantitative methods to determine corproporphyrins in urine introduced in Sweden by Waldenstrom (1), marked an important improvement in the control of lead exposed workers Since the beginning of the 1940 s the principles worked out by Waldenstrom have been successfully applied in the control of lead workers

The Tudor Storage Battery Works in Nol, Sweden, the largest Swedish manufacturer of such batteries employ a total 500—600 workers of which an average of 200 are directly exposed to lead During the period 1941—1960 the control of lead poisoned workers was made by the late professor Martin Odin Experience gained during this period pointed to certain factors as potentially responsible for precipitating lead poisoning in the exposed workers These were

1 *The use of tobacco in the workshop* This applied especially to the use of snuff as snuff taking in Sweden means the introduction of about 5 grams of a tobacco mixture into the oral cavity with a potentially high risk of simultaneous introduction of lead if the hands are not carefully washed Lead contaminated pipe stems were also regarded as a definite risk

2 *Dietary habits* It is known from previous Swedish dietary surveys, Odin (2) Soderberg (3) that those who eat one hot meal per day have a less satisfactory supply of essential nutrients than those who eat several hot meals This could make them more liable to lead poisoning

3 *Leisure time spent indoors instead of outdoors* Several workers were part time farmers owning a small land and spending most of their free time with harvesting and care of animals while others practized fishing cross country running or were active football players The general assumption was that these workers were more seldom lead poisoned

4 *Consumption of alcohol* Notorious alcoholists were known to be liable to lead poisoning In addition to this it was known among the workers at the factory that they should avoid in

sequently been replaced by a 75 cl bottle. Although ten years have elapsed since this partial prohibition ended, most Swedish men still can state their monthly purchase of liquor accurately, and there is no reason to distrust the correctness of the information given.

Liver damage of cirrhosis can result in an elevated urinary excretion of coproporphyrins while the excretion of ALA is normal (Haeger-Aronsen (4), Cramér & Selander unpublished).

The previously assumed high incidence of lead poisoning among regular consumers of liqour could be explained by an elevation of urinary coproporphyrins evoked by alcohol. This does not apply to the excretion of ALA which is not increased during or after acute alcoholism (Cramér & Selander unpublished results). The demonstrated difference in frequency of lead poisoning must therefore be related to some personal factor, not to an interference in the porphyrin metabolism by alcohol. So far it can only be assumed that this factor is lack of personal hygiene or some other type of carelessness on the part of the regular alcohol consumers.

In conclusion, the alcohol consumption of lead workers should be determined before employment. Those who admit that they are regular consumers of strong liquors should be placed in less dangerous position in the factory. Determinations of urinary ALA excretion should be substituted for determinations of coproporphyrins to avoid false diagnoses of lead poisoning.

Summary

Twenty-six pairs of lead workers were examined. In each pair, one worker had shown signs of lead poisoning (ALA output in urine exceeding 1.5 mg/100 ml) and one had not. Time of employment in the factory and degree of lead exposure were comparable.

There was no correlation between smoking and/or snuff taking in the workshop and a higher incidence of lead poisoning. Nor did the leisure time spent indoors affect the frequency of the disease.

Those eating one hot meal per day had an almost statistically significant lower incidence of lead poisoning than those eating several meals.

A definite difference in the incidence of lead poisoning was found between those consuming alcohol regularly (75 cl of strong alcohol per month or more) and those with lower liquor consumption, $\chi^2 = 5.996$, $p < 0.02$.

These results are discussed and some practical conclusions are drawn.

References

1. Waldenström, J.: Sv. Läkartidningen 39: 3151, 1942.
2. Odin, M.: En socialhygienisk undersökning i Västerbottens Län. Kungl. Medicinalstyrelsen, Stockholm, 1934.
3. Söderberg, S.: Socialmed. Tidskrift 36: 143, 1959.
4. Haeger-Aronsen, B.: Scand. J. Clin. Lab. Invest. 12, suppl. 47, 1960.
5. Cramér, K., & Selander, S.: Brit. J. Industr. Med. 22: 311, 1965.
6. Mauzerall, D., & Granick, S.: J. Biol. Chem. 219: 435, 1956.
7. Cramér, K., & Selander, S.: Forhandlinger vid det 13 nordiske yrkeshygiejniske møte, Oslo 1964 (in press).

Table II. *Mean values and ranges for ALA/100 ml urine in 26 lead poisoned and non-poisoned workers*

		Lead poisoned	*Non poisoned*
ALA mg/100 ml	mean value	4.20	0.77
	range	1.80–8.40	0.32–1.35

Table III. *Use of tobacco in working hours*

	None	*Cigarettes*	*Pipe*	*Snuff*
Lead poisoned	10	8	3	5
Not poisoned	11	4	3	8

No significant differences

Table IV. *Hot meals per day*

	1 meal	*2 meals*	*3 meals*
Lead poisoned	6	17	3
Not poisoned	15	9	2

$\chi^2 = 5.112$ $p < 0.05$ (1 meal vs. 2 or 3 meals)

Table V. *Leisure time spent outdoors*

	None	*Only summer*	*The whole year*
Lead poisoned	7	9	10
Not poisoned	4	8	14

No significant differences

Table VI. *Amount of liquor purchased monthly*

	None	*37 cl*	*75 cl or more*
Lead poisoned	8	6	12
Not poisoned	17	6	3

$\chi^2 = 5.996$ $p < 0.02$ (none + 37 cl vs. 75 cl or more)

Discussion

There seems to be no comparable study performed before, and the results are therefore representative only for the factory in question.

One reason for the study was to provide a background for a formal ban on the use of tobacco. No correlation between the use of tobacco and the incidence of lead poisoning was found, however, and it must be postulated that the smokers and snuff takers generally observed the rules of a thorough hand washing before using cigarettes, pipes or snuff.

The difference between those who ate one hot meal and those who ate more than one hot meal per day is contrary to the expected one. It should once more be stressed that the difference lies on the very border line of significance, and that it therefore should not be over emphasized. One possible explanation would be that most of the one meal eaters took their meal in the factory canteen while the others generally ate sandwiches in the day time with a potential danger of simultaneous introduction of lead, owing to a direct contact between the food and more or less lead contaminated hands.

The living habits during leisure time exerted no influence on the incidence to lead intoxication and the previous assumptions of the importance of an abundance of fresh air could not be verified statistically although the figures demonstrate a weak trend in this direction.

The principal finding however is the higher incidence of lead poisoning among those who consumed more than 75 cl alcohol per month. Liquor was rationed in Sweden until 1955 with a monthly ration of 1—3 litres per adult. The one litre bottle has sub

II

PROTEIN DISTURBANCES

II

PROTEIN DISTURBANCES

Rockefeller University New York

Current Trends in Immune Globulin Research

By H G Kunkel J Killander and M Mannik

The essential importance of studies with myeloma proteins and Waldenstrom macroglobulins for elucidating problems of normal immunoglobulins and antibodies is belatedly receiving widespread acceptance It is for this reason that we should particularly honor the individuals who first provided us with these useful tools — Bence Jones as well as his clinical cohorts Watson and McIntyre in 1845 and Professor Waldenström exactly 100 years later

It has become an established sequence of investigative events to first make an observation concerning some characteristic of myeloma proteins or Waldenstrom macroglobulins then to observe the same thing among the normal immunoglobulins and finally to distinguish it as a characteristic of one or another isolated antibody No exceptions have been found concerning the similarity of these proteins and antibodies although it is important to continue to be alert to this possibility

One example might be cited where an observation on a Waldenstrom macroglobulin initiated a most fruitful approach to the study of antibodies This was the observation of Deutsch (1) that these macroglobulins could be readily dissociated with sulfhydryl compounds This was then followed by the finding from several laboratories that 19 S antibodies are inactivated by such compounds and many investigators are now utilizing this sensitivity to distinguish such antibodies However the value of Deutsch s initial observations extended considerably beyond its use among macroglobulins It provided the stimulus that led Edelman in our laboratory to attempt dissociation of γG or 7 S γ-globulin with these compounds This of course led to the definition of the L and H chains and thus markedly influenced the trend of experimental work on the chemistry of antibodies

Many other examples could be given The entire definition of the major classes of immunoglobulins rested primarily on the use of myeloma proteins and Waldenström macroglobulins As Professor Waldenström has so long emphasized these proteins should not be termed paraproteins or pathological proteins In fact they may well be individual antibodies closely resembling the presumed antibody produced by the cell from which the ma

lignant clone developed. Such a concept is supported by the finding of a number of antibody-like activities among Waldenstrom macroglobulins. Cold agglutinin activity and rheumatoid factor activity (2) represent two that are found in a number of instances. Particularly striking has been the case where a Waldenstrom macroglobulin agglutinated old cells (3). In the case of the myeloma proteins as well a few activities have been encountered. Among these is the antistreptolysin activity (4). Another is the property of certain myeloma proteins to combine with lipoproteins, raising the possibility that they might be antibodies to lipoproteins. Certain of these patients show lipid deposits in their tissues which are surrounded by plasma cells, suggesting the possibility of local proliferation of cells reacting in part to the stimulus of perhaps some abnormal lipid material. The problem is complicated by the tendency of certain myeloma proteins to link with a variety of serum proteins such as albumin through disulfide bonds. It is of particular interest that in most instances where antibody-like activities have been encountered in Waldenstrom macroglobulins or myeloma proteins, they have had specificity for some autologous tissue constituents.

Classes of Immunoglobulins and Subgroups

Antigenic studies with myeloma proteins through the use of both rabbit and monkey antisera to such isolated proteins have proven very useful in the *elucidation* of the various classes and *subclasses of immunoglobulins*. The general concensus is that there are now four major classes — γG, γA, γM and γD — which possess very different H chains and similar L chains (see review 5). *In addition, there* appear to be a number of subgroups due to H chain differences for each of the classes. This has been worked out fairly completely for γG globulin where at least four H chain subgroups have been characterized (We or γ_{2b}, Ne or γ_{2a}, Vi or γ_{2c} and Ge or γ_{2d}) (6, 7). Agreement concerning these subgroups has now been obtained in several laboratories.

A major problem in this field is the question of when to call a type of γ globulin a different immunoglobulin or simply a different subgroup, since both classifications depend primarily on antigenic differences in the H chains. Up to now this division has rested primarily on the cross reactions obtained: a protein is a different immunoglobulin if there is no H chain cross reaction and a subgroup if cross reaction occurs, particularly in the Fc fragment. However, this question has become more confused through the recent observations that the different immunoglobulins do cross react partially in the H chains (8, 9). Furthermore, the demonstration of separate genetic factors for the various H chain subgroups of γG globulin emphasizes their basic differences.

Figure 1 illustrates the procedure utilized in the definition of the H chain subgroups of γG globulin as applied to the example of the Vi or γ_{2c}

subgroup Antiserum to a Vi type protein after absorption with most heterologous myeloma proteins of the major We group still reacts strongly with certain myeloma proteins (Ap, Fe and Hu) as well as with a fraction of normal serum and Fr II γ globulin Myeloma protein We and most other myeloma proteins fail to react Approximately 8 per cent of all myeloma proteins show this specific reaction Quantitation of the component in normal γ globulin corresponding to these myeloma proteins has shown considerable variation in different sera in the range of 2—12 per cent of the total γ globulin Immunoelectrophoresis experiments show a short distinct line under the major γG globulin line for all normal sera when unabsorbed antisera to Vi proteins are utilized

The exact chemical basis for the H chain subgroups has not been clearly defined Some carbohydrate differences have been encountered for certain of the subgroups particularly the Ge type where papain digestion reveals a uniquely fast migrating Fc component (6) Reduction with SH compounds indicates marked differences in the availability of the S S bonds that are split This is true for the Vi subgroup where papain digestion is also more rapid and carried further than with the other gamma globulins (10 11) Differences in skin sensitizing capacity have been reported (12)

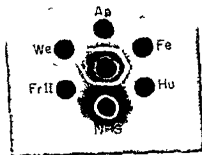


Fig 1 The specific reaction of absorbed anti Vi antiserum with three proteins of the Vi subgroup (Ap Fe Hu) at 0 1 mg/cc as well as the reaction with a similar component in concentrated normal serum and in Fr II at 10 mg/cc concentration A myeloma protein We of a different subgroup is shown for comparison

L Chain Variations

In addition to the well known subdivision of L chains and Bence Jones proteins into the K and L types (Group I and II) further subdivision is readily possible by means of appropriate antisera (13 14) Indications for such variation have long been apparent in our laboratory but have proven difficult to pin down into clearcut subgroups Marked spurs can be demonstrated between different K type proteins with certain antisera At least ten antigenic determinants have been distinguished among the K type proteins through the use of different discriminating antisera (14) All L chains or Bence Jones proteins examined could be distinguished with heterologous antisera as well as homologous antisera showing individual specificity It was clear that more was involved than simple subgroups This antigenic analysis was correlated with structural studies by Hilschmann and Craig on the same proteins (15) and it was apparent that these antigenic differences occurred primarily on the N terminal half of the proteins that is the *portion*

distal to the S S bond linking the L chain to the H chain The antigenic differences were found within Inv(b+) type proteins and within Inv(a+) proteins and could not be correlated with the genetic types A similar situation was found for the type L proteins although here the individual specificity was more marked The results are perhaps best interpreted by assuming a number of different antigenic units which appear in various combinations in different Bence Jones proteins and L chains

Individual Antigenic Specificity and the Variable Areas of the γ-globulin Molecule

Perhaps the most striking feature of the myeloma proteins from the antigenic standpoint is their capacity to elicit antibodies which are specific for the myeloma protein utilized for immunization This property has long been known and has also been described for Waldenstrom type macroglobulins It has received added significance in view of the finding of similar individual specificity for a number of isolated antibodies (16) Table I illustrates the individual specificity for three isolated anti A antibodies The fourth anti A Wa failed to show such specificity and all antibodies were removed by heterologous absorption In all respects these results were similar to those with isolated myeloma proteins

One aspect of this problem that has aroused considerable interest is the question of whether this individual

Table I *Reaction of 28 Isolated Anti A Antibodies with Rabbit Antisera to Four of the Isolated Antibodies*

Isolated Anti A Ab	Antisera to Isolated Anti A Antibodies			
	Anti Th[1]	Anti Ka[1]	Anti Hb[1]	Anti Wa[1]
1 Th	5	0	0	0
2 Wa	0	0	0	0
3 Ha	0	0	0	0
4 Ar	0	0	0	0
5 27	0	0	0	0
6 Ka	0	4	0	0
7 63	0	0	0	0
8 64	0	0	0	0
9 66	0	0	0	0
10 Ho	0	0	0	0
11 Sa	0	0	0	0
12 Hb	0	0	4	0
13—28	0	0	0	0

[1] = Abs with Normal Serum (AB) + Fr II

specificity can be absorbed out by large amounts of pooled γ globulin The concept behind such experiments is that the myeloma proteins have direct analogues in the spectrum of normal γ globulin molecules Most investigations indicated that this was indeed the case However, some results to the contrary have been obtained Recent studies from our laboratories (17) and also by Seligmann and associates (18) indicate that in the vast majority of instances continued absorption with pooled γ globulin causes progressive removal of the specific reactivity This has been brought out best by quantitative precipitin curves with the antisera absorbed with increasing amounts of γ globulin In both laboratories however a few antisera have been encountered which

show little or no diminution of specificity by absorption with pooled γ globulin The explanation for these exceptional findings remains obscure

Considerable interest has focused on the areas of the molecule of the myeloma proteins and isolated antibodies at which the individual specificity resides This area would obviously have important implications regarding the site of structural variation from one antibody to another Initial studies demonstrated that this specificity always resided in the Fab fragment produced by papain (16) which also contained the antibody combining site Further studies (17) with isolated H and L chains primarily from myeloma proteins demonstrated that with some antisera the H chains of the homologous myeloma proteins reacted specifically with others the L chains contained the specific antigenic sites In the case of the H chains this specificity always resided in the Fd fragments and never in the Fc fragments In some instances individual specificity could be localized to both the H and L chains Of particular interest was the frequent finding that individual specificity required the combination of the two chains neither chain alone carried the specificity This was brought out best by recombination of the isolated chains

Thus the localization of the individual *antigenic specificity to the N* terminal half of the L chains and the Fd fragment of the H chains *indicate* that both these areas are subject to great variation from one myeloma protein to another and in all probability from one antibody molecule to another Chemical analyses from finger prints and amino acid sequence studies appear to substantiate these findings although much further work is required particularly for the Fd fragments where only limited studies are available (19)

The Genetic Factors of γG globulins and their Molecular Localization

One very active area of investigation concerning the immune globulins involves the studies of the many genetic factors that have been discovered for these proteins Antisera for the detection of these characters were initially obtained from rheumatoid arthritis patients (20) but at present the more useful source is from sera where isoimmunization has occurred individuals receiving multiple transfusions or immunization by maternal γ globulin Rabbit sera are also finding considerable use particularly in the search for new factors on proteins which at present lack any known genetic determinants (21) The myeloma proteins and Waldenström macroglobulins have played a particularly valuable role in the localization of the genetic factors to the chains of different immune globulins The Inv characters (a b) have been found on the L chains of all classes of immunoglobulins However the known genetic factors of the H chains are thus far limited to γG globulins and even here two of the H chain subgroups lack any known genetic factors It is of special interest that the Gm(a) and Gm(b)

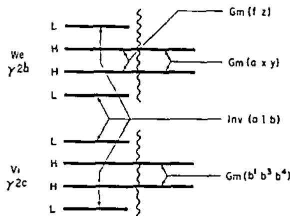


Fig 2 Schematic diagram of the chains of γ globulin for the major We subgroup and the Vi subgroup with the approximate position of Gm and Inv factors on these chains

characters, which have long been known and considered controlled by allelic genes, are found in different H chain subgroups of γG globulin (22) This is illustrated in Figure 2 where the Gm (b_1, b_3 b_4) factors are all localized to proteins of the minor Vi(γ_{2c}) subgroup Gm(a) and (x) occupy a similar position but only in proteins of the major We group

Another finding of interest concerns the localization of Gm(f) and a new factor Gm(z) (23) to proteins of the We group, but to a different site on the H chains (24 25) No factors in this area of the molecule have as yet been discovered for the Vi group but these will undoubtedly be found An extremely interesting picture is evolving concerning the inheritance of these characters which involve structures in different subgroups and different areas of each of the chains of the proteins of the different subgroups It is already apparent that close relationships exist but the number of genetic loci involved is not entirely clear This work will be of undoubted significance for the understanding of the genetic mechanisms involved in antibody synthesis

Interaction of H and L Polypeptide Chains

Another area of active investigation focuses on the antibody combining site and its relationship to the H and L chains The Fab fragments, known to contain the antibody combining site also contain the regions that establish the non covalent interactions between H and L chains Antigen binds primarily to the H chain (26 27, 28), but when the H and L chains are combined through non covalent interactions, the binding activity is enhanced (29 30, 31) These observations may be explained by two major possibilities a) the stable binding site may be formed by two polypeptide chains held together by non covalent interactions b) the binding site may exist only on the H chain but the latter is stabilized by the L chains through non covalent interactions In both of these hypotheses the non covalent interactions between the H and L chains have a relationship to the antibody binding site A relevant question is are the non covalent interactions non specific in that a given H chain may interact with any L chain to produce stable antibody molecules or are the non covalent interactions specific in that a given H chain interacts with a certain L chain to produce a specific stable antibody molecule

The early studies on separated H and L chains of γG globulin demonstrated that L chains effectively solubilized the H chains that tend to aggregate in neutral non dissociating solvents (26) Furthermore even H and L chains from different species can form non covalent interactions to produce four chain molecules (32) indicating considerable affinity between these polypeptide chains The specificity of the non covalent interactions between H and L chains was further studied with H and L chains from individual myeloma proteins (33) The H chains from a γG myeloma protein can effectively combine with L chains from other myeloma proteins to form four chain molecules the recombination is only slightly more effective with homologous chains than with polypeptide chains from two differing proteins However in competitive experiments where the H chains had an equal chance to combine with L chains from the same γG myeloma protein or L chains from another myeloma protein they preferentially recombined with homologous L chains The significance of competition to bring out the preferential combination of homologous H and L chains illustrated in Table II (adapted from 33)

From the table it is apparent that the H chains from the myeloma protein Ge combined effectively with their own L chains and L chains from myeloma protein Ne when the L chains were added individually in only slight molar excess However when both L chains were added simultaneously to the H chains then preferentially the Ge H chains recombined with Ge L chains Similar preferential recombination of homologous H and L chains occurs also in γA myeloma proteins (34)

Table II *Comparison of non competitive and competitive recombination of H and L chains*

H and L chains used for recombination[1]	Per cent of L chains in reformed molecules
Ge H + Ge L	92.3
Ge H + Ne L	85.3
Ge H + Ge L Ne L	Ge 84.0 Ne 4.0
Ne H + Ne L	39.6
Ne H + Ge L	29.8
Ne H + Ne L Ge L	Ne 40.0 Ge 3.0

[1] In all mixtures 0.94×10^{2} micromoles of H chains were used In non competitive mixtures 1.05×10^{2} micromoles of L chains were used in competitive mixtures 1.09×10^{2} micromoles of each of the L chains were used

Several rabbit antibodies have been examined for preferential recombination of H and L chains Roholt and others (35) observed that the H and L chains from antibodies to p azobenzoate and to p azobenzenearsonate recombined preferentially to form the original antibodies when in competition with nonspecific γG L chains as determined by antigen binding activity On the other hand the H chains from rabbit antibodies to the 2 4 dinitrophenyl determinants recombined at random with their own L chains and L chains from nonspecific γG globulin (36) However when hapten was present during recombination in the latter experiments then the antibody H chains preferentially recombined

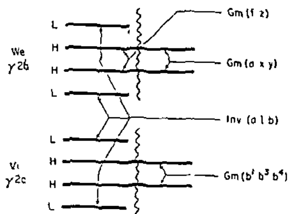


Fig 2 Schematic diagram of the chains of γ globulin for the major We subgroup and the Vi subgroup with the approximate position of Gm and Inv factors on these chains

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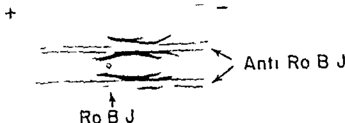


Figure 3 Immunoelectrophoresis experiment illustrating two preparations of Bence Jones protein containing considerable amounts of deficient component recognized with the homologous antiserum

proteins about 10 out of 20 peptides are present in all type L proteins while other peptides are present only in some A number of peptides are unique for each protein as in type K Amino acid analyses reveal consistent differences between type K and L Bence Jones proteins most pronounced for proline alanine valine methionine and phenylalanine (39 40) A number of other interesting findings have come to light recently The Bence Jones proteins usually appear in two different sizes in urine one with molecular weight of about 20 000—25 000 regarded as the monomer and the other the dimer with molecular weight of about 40 000—45 000 Some of the dimers can be split by dissociating solvents such as acid urea or guanidine In some cases however reduction of one disulfide bond is necessary before dissociation can be achieved (37) In a recent report (41) the stable dimer was shown to consist of two monomers linked by a disulfide bond through the cysteine which in type K protein is the carboxyterminal amino acid In Bence Jones proteins type L the corresponding cysteine is next to the carboxy terminal end which is a serine In a stable monomer the cysteine residue is bound to another cysteine to stabilize the sulfhydryl group In the dissociable dimers the two monomers are linked by non covalent bonds In addition to the monomers and dimers there also exist smaller proteins antigenically related to the Bence Jones protein (42 43 44) Recent studies in our laboratory have shown the presence of such components in small amounts in about 30 % of urines with Bence Jones protein of either type K or L (45) They were smaller in size than the monomers and antigenically deficient compared to the Bence Jones proteins and were more readily detected by homologous antisera (Fig 3) In most cases these components were related antigenically to the variable portion of the Bence Jones protein molecule In two cases however they seemed to correspond to the non variable portion

As is the case with the other myeloma proteins the Bence Jones proteins too have a normal counterpart These appear both in the monomeric and the dimeric form (46) and in

with antibody L chains The reasons for these differing observations on rabbit antibodies are not clear at this time, but might well depend on a difference of antibody response to the different haptens employed

Studies on antibodies and myeloma proteins indicate that specificity, probably related to the primary structure, exists in the formation of non covalent bonds between different H and L chains and suggest that variability exists in the structure of the regions participating in these bonds The extent and significance of this specificity are not clearly delineated since four chain molecules are readily formed with "inappropriate" polypeptide chains However experiments on antibodies certainly suggest that "inappropriate" pairing of polypeptide chains does not produce effective specific antibody combining sites

Chemical studies of Bence Jones Proteins

The greatest progress in the chemical analyses of the immunoglobulins has been made with Bence Jones proteins where the partial amino acid sequence is now available for three proteins

Bence Jones proteins excreted in the urine of about a third of patients with multiple myeloma have been shown to be very similar or identical to the light chains of the myeloma protein in the corresponding patients (37) Thus studies of the Bence Jones proteins frequently obtainable in large amounts give valuable information about structural variation in the light chains and can reveal possible correlations between structure and genetic determinants and mechanisms for their synthesis

The partial amino acid sequence of three Bence Jones proteins of type K reported by Hilschmann and Craig (15) and Titani et al (38) is therefore an important step on the way to understand the structure and variation of light chains The sequence in the carboxy terminal half consisting of 106 amino acids of these proteins seems identical with only one exception at position 189 where two of the samples had leucine but the third one had valine In the amino terminal half of the molecule, some parts show similar sequence when two of the proteins are compared but differ in comparison with the third e g the amino terminal peptide with aspartic acid at the N terminal end seems very similar in proteins Ro and Ag while the Cu protein has glutamic acid as the N terminal amino acid and other differences in the amino terminal peptide as well Other parts of the amino terminal half seem unique for each of the proteins such as in the peptide immediately proceeding the nonvariable portion Peptide mapping of several Bence Jones proteins of type K revealed some peptides to occur in all proteins and other peptides to be present in some but not all samples (39) A more extensive comparison has to await the complete sequence of these and several other Bence Jones proteins

Peptide mapping of Bence Jones protein of type L (39) shows no tryptic peptides in common with type K

35 Roholt D A Radzimski G and Pressman, D J Exp Med 122 785 (1965)
36. Metzger H and Mannik, M J Exp Med 120 465 (1964)
37 Gally J A and Edelman G M J Exp Med 119 817 (1964)
38 Titani K Whitley E Avogardo L and Putnam F W Science 149 1090 (1965)
39 Putnam F W and Easley C W J Biol Chem 240 1626 (1965)
40 Van Eijk H C and Monfoort C H Biochim Biophys Acta 86 410 (1964)
41 Milstein C Nature 205 1171 (1965)
42 Deutsch H F Science 141 435 (1963)
43 Deutsch H F Immunochemistry 2 207 (1965)
44 Williams R C Personal communication
45 Solomon A Killander J Grey H M and Kunkel H G In Press Science (1966)
46 Freedman M H and Connell G E Can J Biochem 42 1815 (1964)
47 McKay I and Slater R J J Clin Invest 41 1638 (1962)

crease in excretion after physical exercise (47) Thus also the Bence Jones proteins show no exceptions to the rule concerning the similarity of the "abnormal" proteins and normal immunoglobulins and antibodies It has become abundantly clear that these proteins found in the diseases multiple myeloma and Waldenström macroglobulinemia will form the basis for an exciting chapter of biological research particularly concerning the chemical basis for the variability of the γ globulin molecule which endows it with its unique specificity as an individual antibody

References

1 Deutsch H F Morten J I and Kratochvil C H J Biol Chem 222 39 (1956)

2 Kritzman I J Kunkel H G McCarthy J and Mellors R C J Lab Clin Med 57 905 (1961)

3 Ozer F L and Chaplin H Jr J Clin Inv 42 1735 (1963)

4 Waldenstrom J Winblad S Hallén J and Liungman S Acta Med Scand 176 619 (1964)

5 a) Fahey J I JAMA 194 183 (1965) b) Mannik M and Kunkel H G Immunological Disease Little Brown and Co (1965)

6 Grey H M and Kunkel H G J Exp Med 120 253 (1964)

7 Terry W D and Fahey J L Science 146 400 (1964)

8 Kunkel H G Grey H M and Solomon A Proc IVth Int Cong Immunopath (1965)

9 Mannik M unpublished observations

10 Takatsuki K and Osserman E F Science 145 499 (1964)

11 Poulik M D and Shuster J Nature 204 577 (1964)

12 Terry, W D Proc. Soc Exper Biol and Med 117 901 (1964)

13 Stein S Nachman R L and Engle R L Nature 200 1180 (1963)

14 Solomon A Litwin S D and Kunkel H G Fed Proc 24 201 (1965)

15 Hilschmann N and Craig L C Proc. Natl Acad Science 53 1403 (1965)

16 Kunkel H G Mannik M and Williams R C Science 140 1218 (1963)

17 Grey H M Mannik M and Kunkel H G J Exp Med 121 561 (1965)

18 Seligmann M Proc IV Congr Immunopathology (1965)

19 Frangione B and Franklin E C J Exp Med 122 1 (1965)

20 Grubb R and Laurell A B Acta Path and Microbiol Scand 39 390 (1956)

21 Litwin S D and Kunkel H G In Press Transfusion (1965)

22 Mårtensson L and Kunkel H G J Exp Med 122 799 (1965)

23 Litwin S D In Press Nature (1966)

24 Kronvall G Vox Sang 10 303 (1965)

25 Steinberg A G Cold Spring Harbor Symposium (1969)

26 Fleischman J B Pain R H and Porter R R Arch Biochem and Biophysics Suppl 1 174 (1962)

27 Fleischman J B Porter R R and Press E M Biochem 88 220 (1963)

28 Utsumi S and Karush F Biochem (ACS) 3 1329 (1964)

29 Franek F and Nezlin R S Folia Microbiol (Prague) 8 128 (1963)

30 Edelman G M Olins D E Gally J A and Zinder N D Proc Natl Acad Sci 50 759 (1963)

31 Roholt D A Onoue K and Pressman D Proc Natl Acad Science 51 173 (1964)

32 Olins D E and Edelman G M J Exp Med 119 789 (1964)

33 Grey H M and Mannik M J Exp Med 122 619 (1965)

34 Prendergast R A Grey H M and Kunkel H G Fed Proc 24 615 (1965)

Fig 1 To illustrate the reaction between anti pepsin split γG globulin antiserum (R 61) and various γG globulin preparations

f en ral b ts Three of these (R 150 R 151 R 152 were immunized with a pepsin split G eloma protein of type K three (R 153 R 154 R 155 w h a pepsin split G myeloma pro of type L and the remaining f e an als (R 60 R 61 R 167 R 168 R 169 with pepsin split normal γG globulin The initial immunization course consisted of three week l injections of f e m protein n complete Freund s adjuvant followed by three similar injections of prot n n s ne The animals w e k lled one w k after the last injection. b o ter inje t ns consisted of peps split γG globulin n saline Serum samples were ol a l o er a n ne mon h period They w all t sted as descr b d below

I nolog c l techniques Immunoelectro p o s a d double diffus n te ts n agar re performed as described elsewhere (1 The (1 Cn 4 and Gm 5 types were d 1 1) the f low ng reagents f r (l r 1 c lls coat l w l an D 88 and the agglutinat r Smej a f r Gm(4 ant) \ \ 4 a l f r G 5 an D Bl \ erud f a l ac og l u aen a s ru 1 to be te ted a ulj ct d o l n ty grad e t ultracen ri f al l rac o s wh 1 con a ned γM glo bul a d γG gl bul n as judged fro the pro cur e were pool l a d d alysed

against saline before testing From the same tubes the fractions containing maximal concentrations of γG globulin were pooled and treated in the same way Isolated γM globulin was tested for inhibiting capacity both in the intact state and after reduction and alkylation

Experiments and results

Characterization of antisera

All antisera were tested in the same way and gave similar results By immunoelectrophoresis against whole human serum a single precipitin line was seen with the characteristic position and shape of γG globulin Immunoelectrophoresis against papain split γG globulin showed that the antisera precipitated only with the Fab fragment Control experiments with anti γG globulin antisera showed that these gave strong precipitin lines with both the Fab and Fc fragments

Double diffusion tests where the antisera reacted against intact γG globulin and pepsin split γG globulin showed reactions of identity in all instances No spurs were seen after up to three days incubation whereas control anti γG globulin antisera produced marked spurs after 24 hours incubation under identical conditions Similar tests where the reactions of pepsin split γG globulin and reduced and alkylated pepsin split γG globulin were compared again showed reactions of identity in all instances The appearance of these reactions are illustrated in Figure 1

It was concluded *from these* experiments that all the antisera were speci

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Structural relationship between γG- and γM-globulin in man

By MORTEN HARBOE and JOHANNA DEVERILL

Exposure of γG globulin to papain at pH 7 4 leads to formation of antigenically distinct fragments, the Fab (slow) and Fc (fast) fragments (6) Structures within the Fab fragment are also present in γM globulin whereas the Fc fragment is specific for γG globulin (11 18) Immunological (4 8 22), biochemical (2 3 4 5) and genetical (12 16) studies have shown that the light polypeptide chains of γG globulin which are present within the Fab fragment are common to γG and γM globulin The Fab fragment also contains a part of the heavy chain (10, 27) It is not clear whether this part of the heavy chain the Fd fragment is common to the two proteins This question is of particular interest since it is generally thought that the antigen binding sites are associated with this part of the heavy chain

The present paper describes experiments during which antigenic determinants characteristic of the Fd fragment of γG globulin were demonstrated in γM globulin

The nomenclature used for genetic factors of human immunoglobulins is described in Bull Wld Hlth Org 33 721 1965

Materials and Methods

γG globulin preparations Normal γG globulin was a commercial Cohn Fr II preparation (AB Kabi Stockholm Sweden) G myeloma proteins were purified by zone electrophoresis using Pevikon as supporting medium (23) *Splitting with papain* (papain 2× crystallized Worthington Biochemical Corp Freehold N J) and pepsin (pepsin 2× crystallized Worthington Biochemical Corp) was carried out as previously described (28) Tests for Gm(1) activity were used to control that digestion with pepsin was complete (15 28) Light and heavy polypeptide chains of γG globulin were prepared as described elsewhere (9 17)

γM globulin preparations γM globulin was highly purified from sera of patients with typical Waldenstrom s macroglobulinaemia by a two step procedure Depending on the physico chemical properties of the individual macroglobulin the initial purification step was either zone electrophoresis or precipitation by dilution of serum with distilled water The macroglobulin preparations thus obtained were further purified by density gradient ultracentrifugation (21) The protein was reduced and alkylated as described by Fleishman et al (9)

Immunization procedure Antisera against pepsin split γG globulin were collected from

Demonstration of precipitin reaction between anti Fd antisera and γM globulin

Two of the anti Fd antisera (obtained from rabbit 167 and 169) precipitated with most but not all of eleven highly purified γM globulins in gel diffusion tests that are illustrated in figure 2 The strength of the reaction varied markedly within the group of precipitating macroglobulins even though the same concentration (1 mg/ml) of γM globulin was used in all instances

Nine of the anti Fd antisera failed to precipitate purified γM globulins in similar tests

Tests for Gm(4) in γM globulins

The Gm(4) factor remains intact after pepsin splitting of γG globulin (13 19) and the determining site has been localized to the heavy chain (13) The Gm(1) and Gm(a) factors are destroyed by pepsin splitting of γG globulin (28) Their determining sites are located on the Fc fragment of γG globulin (12 16) and the factors are only present on this protein (7 12 16 24)

Thirteen macroglobulinaemia sera were tested for these factors in isolated γG and γM globulin fractions The tests for Gm(1) and Gm(a) were performed to control the purity of the γM globulin preparations All γM globulins were Gm(-1 -a) whereas the γG globulin fraction of the same sera was positive for at least one of these factors in all instances In 13 sera the isolated γG globulin was Gm(4) whereas the isolated γM globulins were Gm(-4)

Discussion

Rabbit antisera against human γG globulin react with different antigenic determinants on this molecule A major part of the antibodies usually react with antigenic determinants on the Fc part of the molecule this reaction is specific for γG globulin Part of the antibodies react with antigenic determinants on the light chains These antibodies cross react with γM globulins (4 8 11 18 22) Biochemical (2 3, 4 a) and genetical (12 16) studies supplement these findings indicating that the light chains are identical in γG and γM globulins The Fd fragment of human γG globulin has been less studied because of its low antigenicity

Pepsin splitting of human γG globulin results in the formation of a fragment that is very similar to the Fab fragment obtained by papain splitting at pH 7 4 (25 28) It was found that immunization of rabbits with pepsin split γG globulin resulted in the production of potent antisera that reacted with the Fab fragment whereas no reaction with the Fc fragment was observed in the precipitation tests used in this study After absorption with isolated light chains from normal γG globulin the antisera still precipitated with the isolated heavy chains (γ chains) These findings indicated that the absorbed antisera reacted with the part of the heavy chain contained within the Fab fragment the so called Fd fragment and they are referred to as anti Fd antisera

From the studies of the reaction of anti Fd antisera with highly puri

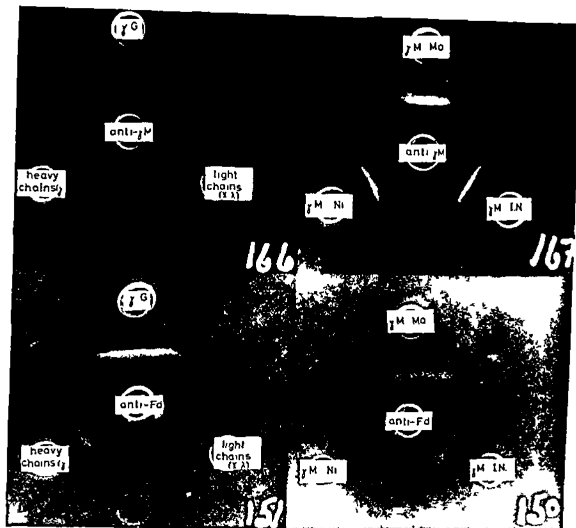


Fig 2 To show that anti Fd antiserum (R 167) precipitated with some but not all γM globulins For further explanation see text

fic for the Fab fragment of γG globulin when tested by these gel diffusion techniques All the antisera cross reacted with highly purified γM globulin and thus appeared useful for further study of the basis of the cross reaction between γG and γM globulin

Reaction of the antisera with isolated polypeptide chains of γG-globulin

All the antisera produced against pepsin split γG globulin precipitated with isolated light and heavy chains of γG globulin After absorption with isolated light chains in antigen excess they still precipitated with isolated heavy chains of γG globulin (γ chains) It was concluded that the absorbed antisera only reacted with the Fd part of the γG globulin molecule and they are referred to as anti Fd antisera They precipitated intact γG globulin pepsin split γG globulin and isolated chains with reactions of identity

enzymatic splitting was not detectable in 13 purified "monoclonal" γM globulins even though the factor was demonstrated in the γG globulin fraction of all these sera

Acknowledgements

We wish to thank L Mårtensson for provision of anti Gm (4) serum This work was aided by grants from the Norwegian Council on Cardiovascular Disease The Norwegian Council for Science and The Humanities and The Nansen Foundation

References

1 Ballieux R E Bernier G M Tominaga K & Putnam F W Science 145 168 1964
2 Carbonara A O & Heremans J F Arch Biochem 102 137 1963
3 Chaplin H Cohen S & Press E M Biochem J 95 256 1965
4 Cohen, S Biochem J 89 334 1963
5 Edelman G M Ann N Y Acad Sci 101 246 1962
6 Edelman G M Heremans J F Heremans M Th & Kunkel H G J exp Med 112 203 1960
7 Fahey J L & Lawler S D J nat Cancer Inst 27 973 1961
8 Fahey L & Solomon A J clin Invest 42 811 1963
9 Fleischman J B Pain R H & Porter R R Arch Biochem suppl 1 174 1962
10 Fougereau M & Edelman G M J exp Med 121 373 1965
11 Franklin E C & Stanworth D R J exp Med 114 521 1961
12 Franklin E C Fudenberg H Meltzer M & Stanworth D R Proc nat Acad Sci Wash 48 914 1962
13 Gold E R Mandy W J & Fudenberg H H Nature Lond 207 1099 1965
14 Grey H M & Kunkel H G J exp Med 120 253 1964
15 Harboe M Acta path microbiol scand 47 199 1959
16 Harboe M Osterland C K & Kunkel H G Science 136 979 1962
17 Harboe M, Deverill, J & Godal H C Scand J Haemat 2 137 1965
18 Heremans J F & Heremans M Th Acta med scand 170 (suppl 367) 27 1961
19 Kronvall G Vox Sang 10 503 1965
20 Kunkel H G cited in (26)
21 Kunkel H G Rockey J H & Tomasi T Methods of separation and properties of antibodies of high molecular weight p 30 in Immunochemical Approaches to Problems in Microbiology Eds M Heidelberger and O J Plescia Rutgers University Press New Brunswick N J 1960
22 Mannik M & Kunkel H G J exp Med 116 859 1962
23 Muller Eberhard H J Scand J clin Lab Invest 12 33 1960
24 Mårtensson L Acta med scand 170 (suppl 367) 87 1961
25 Nissonoff A Wissler F C Lipman L N & Woernley D L Arch Biochem. 89 230 1960
26 Nussenzweig V & Benacerraf B Immunopathology Information Exchange Group no 45 1965
27 Olins D E & Edelman G M J exp Med 116 635 1962
28 Osterland C K Harboe M & Kunkel H G Vox Sang 8 133 1963
29 Takatsuki K & Osserman E F Science 145 499 1964
30 Terry W D & Fahey J L Science 146 400 1964

fied γM globulin, two interpretations emerge Firstly, two of the eleven anti-Fd antisera precipitated with highly purified γM globulins (cf Figure 2) These findings show that antigenic determinants in the Fd fragment of γG globulin are also present in γM-globulin, or in other words that more than the light chains are common to these proteins Similar findings have been made by Nussenzweig and Benacerraf (26) in studying guinea pig 7S γ_1, and 7S γ_2 globulins These two proteins have distinct antigenic determinants in the Fc part of the molecules but share common structures both in the light chains and in the Fd fragment Secondly, several findings indicate that the structural relationship between the Fd fragment of γG globulin and γM globulin is very complex i) Nine of our anti Fd antisera did not precipitate isolated γM globulin ii) Two of our anti-Fd antisera precipitated some but not all of the 'monoclonal γM globulins tested Similar findings have been made by Kunkel (20) It was also evident that the strength of the precipitinlines varied considerably within the group of precipitating γM globulins iii) The division of "monoclonal γM globulins into a precipitating and a non precipitating group according to their reactions with these two anti Fd antisera did not correspond to the antigenic heterogeneity recently described in the heavy chain of γM globulin (17) iv) The Gm(4) factor that is intact in the Fab fragment after enzymatic splitting of γG globulin is not present in γM globulin as shown above and in an independent study (13)

Antigenically distinct subgroups of the heavy chain of γG globulin have been described recently (1, 14, 29 30) Heterogeneity of the heavy chain of γM globulin has been demonstrated by similar techniques (17) Further studies of these forms of heterogeneity may help in delineating the complex structural relationship between the Fd fragment of γG globulin and γM globulin Such information would be of great interest since this is the part of the γG globulin molecule that is involved in antigen binding Little is known to date about the structure of antigen binding sites with presumed identical serological specificity in γG and γM globulin Such information is needed to obtain a better understanding of the basic mechanisms involved in antibody production

Summary

Antisera produced in rabbits against human pepsin split γG globulin reacted with both the heavy and light polypeptide chains of γG globulin After absorption with isolated light chains in antigen excess they reacted only with the Fd part of this molecule

Two out of eleven such antisera precipitated with some but not all of eleven highly purified "monoclonal" γM globulins These findings show that antigenic determinants in the Fd fragment of γG globulin are also present in γM globulin

The Gm(4) factor that is intact in the Fab fragment of γG globulin after

genic class of heavy and more than one antigenic class of light polypeptide chains

It is therefore not surprising that the untypable M protein offers a challenge for further study and may often provide the first clue to an interesting and novel disorder of protein synthesis This is strikingly illustrated by two recent experiences In one instance failure of a protein to type for k or λ chains led to the suspicion and subsequent description of the first patient with so called "Heavy Chain disease" a disorder associated with the production of Fc fragments (8) In another failure of a protein to react with antisera to α γ and μ chains resulted in the discovery of a new immunoglobulin class IgD (9)

Another and possibly quite heterogeneous group of untypable myeloma proteins are the M proteins which possess one of the heavy chains but which cannot be typed for their k and λ chain in the intact state The first two such proteins were clearly shown to possess one type of light chain after reduction and alkylation and were thought to represent a myeloma protein with an unusual tertiary structure possibly containing hidden or inaccessible light chains (10 11) We have recently encountered another such homogeneous protein which represents yet another mechanism

The protein initially isolated from the serum had the following properties It had the electrophoretic mobility of a γ globulin had a sedimentation coefficient of 3 8S at infinite dilution and belonged to the γ b (We) subtype of γ chains While the native protein failed to react with antisera to k and λ chains light chains prepared by reduction and alkylation strongly reacted with antisera to the k Type Reduction and alkylation yielded the expected amounts of heavy and light chains and peptide maps of these and the isolated Fc and Fab fragments produced after addition of papain were indistinguishable from other We myelomas having Type k light chains (12)

Further studies revealed that the protein and the Fab fragment failed to precipitate with antisera to k chains but that both inhibited the precipitation of light chains by antisera to k chains both in quantitative precipitation tests and by causing visible shortening of the precipitin line in double diffusion analyses Only detailed immunologic studies after completion of these initial experiments demonstrated that the material responsible for this behavior represented the products of complete spontaneous degradation which resembled in all respects the 3 5S fragments produced by papain digestion A subsequent bleeding from this patient yielded a classical 7S γG myeloma belonging to the We subtype and which was easily typed as belonging to the k antigenic class

The reason why this protein could not be readily classified becomes obvious on the basis of previous studies (13) which demonstrated that light chains may behave as *univalent* antigens when tested with certain antisera Bence Jones Proteins precipitate with

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The Problem of the Untypable M Protein [1]

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The Problem of the Untypable M Proteins

Studies of myeloma proteins and especially macroglobulins since their discovery by Professor Waldenstrom in 1945 (1), have yielded much information about the corresponding normal immunoglobulins which are often present in low concentrations and, consequently, difficult to isolate from normal serum

Antigenic properties characteristic of the different polypeptide chains found in the immunoglobulins have permitted the production of a variety of antisera useful in characterizing the homogeneous protein components (M proteins) associated with a number of proliferative disorders of plasma cells and lymphocytes Antisera specific for the α, γ and μ heavy chains generally allow classification of such proteins into the IgA, IgG or IgM class respectively (2, 3), while antisera to k and λ chains permit further identification of the light chains as belonging to one of the two recognized classes (4, 5) More recently antigenic analyses have resulted in the discovery of at least four subtypes of γ chains which allow even more precise typing of G myeloma proteins (6 7) The ease of performing such immunologic analyses has resulted in the widespread use of these techniques in the classification of M proteins and it seems likely that had Professor Waldenstrom encountered his first patient with macroglobulinemia twenty years later he would have suspected the disorder on the basis of such immunologic tests

Extensive studies of large numbers of M proteins have led to the conclusion that BJP s belong either to the k or λ type and that the larger serum proteins contain one class of heavy chains and one of the light chain types None of these proteins has been shown to contain more than one anti

[1] Supported by New York City Health 8527 and 1431 p and by Cancer Research Research Council USPHS Grants # AM 2594 Funds of the University of California and Grant # T 356 from the American Cancer Society

3 Heremans J Les Globulines Seriques du Systeme γ Ed Arscia Brussels 1960
4 Mannik M & Kunkel H G J Exp Med., 117 213 1963
5 Fahey J L J Immunol 91 448 1963
6 Grey H & Kunkel H G J Exp Med., 120 253 1964
7 Terry W & Fahey J Science 146 400 1964
8 Franklin E C Lowenstein J Meltzer M & Bigelow B Am J Med., 37 332 1964
9 Rowe D S & Fahey J L J Exp Med 121 171 1965
10 Rowe D S & Fahey J I J Exp Med., 121 185 1965
11 Osterland K C & Chaplin H Fed Proc. 24 No 2 502 1965
12 Frangione B & Franklin E. C. J Exp Med., 122 1 1965
13 Franklin E C. J Immunol., 91 730 1963
14 Palmer J L & Nisonoff A J Biol Chem 238, 2393 1963

such antisera because they normally exist as dimers, while the Fab fragment, which contains only a single light chain, fails to precipitate, but can inhibit precipitation of the appropriate BJP by combining with the antibody. In contrast, the intact IgG and the 5S pepsin F(ab)'₂ fragment, each of which has two identical light chains, are precipitated by such antisera.

Based on this finding, M proteins, which can be typed for their L chain only after isolation and dimerization of their L chains but not in the intact state, may have the following structures:

1. They may be half molecules of the type produced in vitro by Nisonoff and Palmer (11). Such molecules could be distinguished from 7S IgG only by molecular weight determinations since they would have an amino acid composition identical to IgG but would probably not precipitate with anti light chain sera. It was initially thought that this patient may have produced such a protein.

2. They may have two identical light and two identical heavy chains but one of the light chains may be buried or otherwise inaccessible and thus unable to react with the antiserum. Such a model could be established only by exclusion if a normal molecular weight is found (10).

3. They may represent free Fab fragments, either synthesized as such or products of spontaneous degradation, as was probably the case in our patient.

4. Alternatively, such a molecule may consist of two dissimilar light chains, one of Type K and one of Type λ. Since it has been clearly shown that K and λ chains are not produced in the same cell, this possibility is not very likely.

If one were to rule out the fourth possibility, a choice between the other three would rest upon precise molecular weight calculations and careful antigenic analyses.

In addition to the above described possibilities, the untypable M protein, when tested with the usual antisera to heavy and light chains may represent as yet unrecognized disorders of protein synthesis. Thus, new classes of light chains and heavy chains may be discovered as was the case with IgD (9). In addition, proteins not reactive with antisera to light chains may represent "heavy chain" (Fc fragment) disease related to the α and μ chains. Finally, if the six chain model of γ globulin shall prove to be correct, Fd fragments only may be produced by certain patients and these would not be expected to react with antisera to light chains or the Fc fragment determinants of heavy chains. Thus, it is obvious that careful study of all untypable M proteins is warranted and that such studies may uncover examples of hitherto undescribed disorders of protein synthesis.

References

1. Waldenström J. Acta Med Scand 117: 216, 1944.
2. Franklin E. C. & Kunkel H. G. J Immunol 78: 11, 1957.

3 Heremans J Les Globulines Seriques du Systeme γ Ed Arscia Brussels 1960
4 Mannik M & Kunkel H G J Exp Med 117 213 1963
5 Fahey J L J Immunol 91 448 1963
6 Grey H & Kunkel H G J Exp Med 120 253 1964
7 Terry W & Fahey J Science 146 400 1964
8 Franklin E C Lowenstein J Meltzer M & Bigelow B Am J Med 37 332 1964
9 Rowe D S & Fahey J L J Exp Med 121 171 1965
10 Rowe D S & Fahey J L J Exp Med 121 185 1965
11 Osterland K C & Chaplin H Fed Proc 24 No 2 502 1965
12 Frangione B & Franklin E C J Exp Med 122 1 1965
13 Franklin E C J Immunol 91 730 1963
14 Palmer J L & Nisonoff A J Biol Chem 238 2393 1963

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Biological significance of exocrine gamma-A-immunoglobulin

By JOSEPH F HEREMANS, PAUL A CRABBE and PIERRE L MASSON

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Gamma-A-immunoglobulin (*also called IgA gamma 1A or beta 2A*) is a molecular variety of antibody active proteins, which was first isolated and characterized by one of the present writers during work on myeloma proteins at Professor Waldenstrom's laboratory in the summer of the year 1957 (18) It represents about 20 per cent of the total circulating immunoglobulins, and investigations from various sides have demonstrated that it is the carrier of a considerable spectrum of antibody activities (rev cf ref 17) In this respect it seems not inferior to the more abundant form of immunoglobulins *viz* gamma G (formerly called 7S gamma) Therefore the question may be asked why the organism should find it advantageous to multiply the molecular species effecting identical antigen combining tasks, in other words why gamma A should exist at all

To discuss this point it may be profitable to consider the following facts and concepts which have emerged out of old and recent research (1) the distribution of gamma A in various biological fluids, and (2) the concept of *exocrine antibodies*

(1) *Distribution of gamma A immunoglobulins in various biological fluids*

Although the concentration of gamma A in normal plasma is only about one quarter of that of gamma G the situation is very different in all biological fluids that are not mere transudates of the plasma Thus the concentration of gamma A much exceeds that of gamma G in milk and colostrum (14) saliva (22 25 33) tears (5) nasal secretions (29) bronchial secretions (21 24 28) as well as in secretions from villous tumours of the rectum (27) Notwithstanding the fact that large amounts of plasma proteins continuously leak into the digestive tract the gamma A gamma G ratio in the fluid obtained by intestinal aspiration is higher than the corresponding ratio in the serum (5) and the same is observed in bile (5) Some kind of lo

cal production of gamma A must therefore be assumed at all such sites unless one is willing to admit a preferential transfer of gamma A from the plasma

The latter question now appears to be solved The gamma A present in exocrine secretions is obviously largely generated in the glands or surfaces responsible for their production and not derived from the plasma This is demonstrated by the failure of intravenously injected ^{131}I labelled gamma A to appear in the parotid fluid (32) as well as by the fact that salivary glands (20 32) mammary glands (20) and intestinal mucosa (2) incorporate labelled amino acids into gamma A immunoglobulin The connective tissue stroma of all these structures is richly endowed with plasma cells the accepted sources of immune globulins For several years our group has been engaged in an immunohistochemical study of those mucosal and glandular plasma cells with the aim of verifying the nature of the immunoglobulins they contain It has thus been shown that the lamina propria of the duodenum and upper part of the jejunum numbers 350 000 gamma A containing cells per cubic mm on the average against only 15 000 gamma G containing cells and 51 000 gamma M containing cells per cubic mm (6 7) A typical field of lamina propria surrounding the crypts of Lieberkuhn and displaying a wealth of plasma cells filled with gamma A is illustrated in Fig 1 Similar findings have been made on other sections of the gut (9)

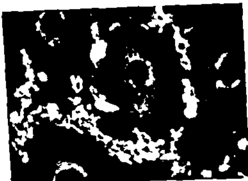


Fig 1 Plasma cells containing gamma A immunoglobulin in a normal human intestinal mucosa Frozen section through the crypts of Lieberkuhn of a duodenal biopsy stained with fluorescein labelled antiserum against gamma A immunoglobulin The plasma cells form dense sheaths around the crypts

as well as on the stroma of salivary glands and of the bronchial mucosa

Whereas the bulk of gamma A present in secretions is thus demonstrably independent from any supply by the plasma it may conversely be asked to what extent such local production of gamma A may contribute to the circulating pool of this protein Indirect evidence pleads against the assumption that such a contribution from exocrine sources would be significant The exocrine type of gamma A displays one significant physicochemical difference with respect to its counterpart in the plasma *viz* its high molecular weight This has been demonstrated for the gamma A from milk and saliva (32) as well as for that from bronchial secretions (26) In all such secretions the bulk of the gamma A sediments in the ultracentrifuge as a heterogeneous population of components chief of which are those with

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Biological significance of exocrine gamma-A-immunoglobulin

By JOSEPH F HEREMANS, PAUL A CRABBE and PIERRE L MASSON

Joseph F Heremans Professor of Internal Medicine at the University of Louvain (Leuven) Belgium

Gamma-A-immunoglobulin (also called IgA, gamma 1A or beta 2A), is a molecular variety of antibody active proteins which was first isolated and characterized by one of the present writers, during work on myeloma proteins at Professor Waldenström's laboratory in the summer of the year 1957 (18) It represents about 20 per cent of the total circulating immunoglobulins, and investigations from various sides have demonstrated that it is *the carrier of a considerable spectrum of antibody activities* (rev cf ref 17) In this respect it seems not inferior to the more abundant form of *immunoglobulins viz* gamma G (formerly called 7S gamma) Therefore *the question may be asked why the organism should find it advantageous* to multiply the molecular species effecting identical antigen combining tasks *in other words why gamma A should exist at all*

To discuss this point it may be profitable to consider the following facts and concepts which have emerged out of old and recent research (1) the *distribution* of gamma A in various biological fluids and (2) the concept of *exocrine antibodies*

(1) Distribution of gamma A immunoglobulins in various biological fluids

Although the concentration of gamma A in normal plasma is only about one quarter of that of gamma G the situation is very different in all biological fluids that are not mere transudates of the plasma Thus the concentration of gamma A much exceeds that of gamma G in milk and colostrum (14) saliva (22 25 33) tears (5) nasal secretions (29) bronchial secretions (21 24 28) as well as in secretions from villous tumours of the rectum (27) Notwithstanding the fact that large amounts of plasma proteins continuously leak into the digestive tract the gamma A gamma G ratio in the fluid obtained by intestinal aspiration is higher than the corresponding ratio in the serum (5) and the same is observed in bile (5) Some kind of lo

cal production of gamma A must therefore be assumed at all such sites unless one is willing to admit a preferential transfer of gamma A from the plasma

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sedimentation rates of 9 S and 11 S In contrast, the gamma A immunoglobulin from normal human serum is mainly present under the form of a monomer having a sedimentation coefficient of about 7 S A small proportion of polymers, accounting for 10—15 per cent of the total (15, 16) is also present in the serum, and does not seem to be an artifactual aggregation product ascribable to the method of isolation Therefore, if part of the exocrine gamma-A is assumed to reach the bloodstream, it can hardly account for more than one tenth, possibly less, of the total circulating pool of gamma A

(2) The concept of exocrine antibodies

In 1922 attention was drawn by Davies (10) on the fact that stools from patients with bacillary dysentery often contained agglutinating antibodies against *Shigella*, in the absence of demonstrable agglutinating activity in the serum Experimental work with cholera vaccine, administered by way of the mouth or the stomach in guinea pigs (1) and the human (12 13) has led to the concept that a particular class of antibodies, called 'coproantibodies" are synthesized by the digestive tract upon local contact with the antigen Such antibodies appear to have distinct biological properties their titers in the secretion reach an earlier and higher maximum but wane faster than the corresponding titers of circulating antibodies directed against the same antigen Antibodies with similar behaviour have also been raised in the human female genital tract upon intravaginal application of an antigen (31), and the local production of antibodies in the udder of lactating cows upon intramammary introduction of antigens is an established knowledge in veterinary medicine We may therefore assume that the above described properties are not restricted to "copro antibodies" or "mucosal antibodies', but are characteristic for exocrine immunoglobulins in general

In view of the unique predominance of gamma A immunoglobulin in all types of exocrine secretions, and considering that the overwhelming majority of the subepithelial plasma cell population is obviously engaged in the production of precisely this type of immunoglobulin, one cannot escape the conclusion that exocrine gamma A immunoglobulin and exocrine (mucosal) antibody must be one and the same thing

If this equation has any meaning we may now turn to the question as to why a special type of immunoglobulin should be required to serve as an exocrine antibody Perhaps the answer is twofold

In the first place there is one physicochemical property of gamma A that seems to make it eminently suitable for such a role To resume a term coined by Burnet (3) the variety of local antibody here discussed should be viewed as an "antiseptic paint" Now paints are by definition endowed not only with coating but also with adhesive properties This is precisely the case with

gamma A, which has an unusual tendency to form complexes with the most diverse kinds of other proteins (18) One may even guess that the same property allows it to act as a skin sensitizing antibody i e as a carrier of allergic reaginic activity (11 19 23)

There is however another aspect to the point The antibodies synthesized by the lamprey, a form of *Chordata* even more primitive than the earliest vertebrates, appear to have molecular weights intermediate between those of gamma G (7 S) and gamma M (19 S) antibodies but falling in the range of the polymeric forms of gamma A encountered in exocrine secretions (1) Now lampreys do not possess any well defined lymphoid organs such as lymph nodes their intestinal mucosa however rests on a connective tissue stroma whose lymphoid cell population is much reminiscent of that in man It would not be surprising therefore if gamma A eventually turned out to be an ancestral type of immunoglobulin formerly exclusively associated and presently still largely restricted to production sites close to the epithelial surfaces by which the organism establishes contact with the septic environment of the outside world

If the latter assumption proves true then gamma A might be the living fossile among the immunoglobulins and evolution may be expected to discard it sooner or later Perhaps this is what is actually being experimented already in those extremely rare individuals who in spite of a perfect health



Fig 2 Duodenal biopsy from a patient with idiopathic steatorrhoea associated with a severe deficiency of gamma A immunoglobulin in the serum and in all secretions Only a few fluorescent plasma cells are visible in this section which has been stained with fluorescein labelled antiserum against gamma A

seem to be entirely devoid of gamma A immunoglobulins (30) Still some aspects of the function of gamma A as exocrine antibody may nevertheless remain of importance This is suggested by the discovery of a curious syndrome (8) in which idiopathic steatorrhoea is associated with an apparently very important lack of gamma A immunoglobulins in the serum the exocrine secretions and the plasma cells of the intestinal wall (Fig 2)

Summary

Gamma A immunoglobulin is found in much higher relative concentrations in all exocrine secretions than in the serum

Two interpretations are given for this observation. It would seem that gamma A is the material substrate for

sedimentation rates of 9 S and 11 S. In contrast, the gamma-A immunoglobulin from normal human serum is mainly present under the form of a monomer having a sedimentation coefficient of about 7 S. A small proportion of polymers, accounting for 10—15 per cent of the total (15, 16) is also present in the serum, and does not seem to be an artifactual aggregation product ascribable to the method of isolation. Therefore, if part of the exocrine gamma-A is assumed to reach the bloodstream, it can hardly account for more than one tenth, possibly less, of the total circulating pool of gamma-A.

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In view of the unique predominance of gamma-A immunoglobulin in all types of exocrine secretions, and considering that the overwhelming majority of the subepithelial plasma cell population is obviously engaged in the production of precisely this type of immunoglobulin, one cannot escape the conclusion that exocrine gamma-A-immunoglobulin and exocrine (mucosal) antibody must be one and the same thing.

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In the first place there is one physicochemical property of gamma-A that seems to make it eminently suitable for such a role. To resume a term coined by Burnet (3), the variety of local antibody here discussed should be viewed as an "antiseptic paint". Now paints are by definition endowed not only with coating but also with adhesive properties. This is precisely the case with

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Sedimentation Constants of IgG and IgD Myeloma Proteins Compared with those of Normal IgG

By Ulla Britt Hansson C B Laurell and R Bachmann

In 1965 Rowe and Fahey (9) described the characteristics of the new class of immunoglobulins IgD One isolated IgD myeloma protein was found to have a sedimentation constant ($s_{20,w}$) of 7 0 against 6 6 for normal IgG Of our series of 1 300 sera with M components four were identified as IgD Three of these samples were still large enough to allow isolation of the M component and estimation of the sedimentation constants Their constants were lower than or the same as our constants for normal IgG We therefore compared the sedimentation constants of some isolated M components of types IgG and IgD with those of some isolated individual normal immunoglobulins

Material and methods

Frozen sera with M components of type IgD number 510 793 and 10 4) and of type IgG (number 165 909 8, 34 and 226) stored at −15 C for one to several years

Fresh serum with M components of type IgG number 1311)

Fresh normal individual sera (number 1 to 8

Paper electrophoresis was run in barbital buffer 0 05 M containing 2 mmol CaCl

Isolation of M component (IgG and IgD) and of normal IgG For the studies 0 25 to 0.30 g IgG (IgD) was used One part serum was diluted with three parts 0 9 % NaCl and ammonium sulphate was added to a final concentration of 1 84 M with stirring at room temperature The pH was adjusted with ammonia to 7 After two hours at +4° C the precipitate was centrifuged down, washed resuspended three times in ammonium sulphate (1 84 M) at pH 7 and +4° C and dissolved The sample was dialysed first for at least 2 hour against running tap water and then over night against 0 9 % NaCl and finally for at least 5 hours against 0 02 M phosphate buffer pH 8 0 The material was fractionated on a DEAE column using a salt gradient with increasing ionic strength and decreasing pH (400 ml 0 02 M phosphate buffer pH 8 0+400 ml 0 2 M phosphate buffer pH 6) The eluate containing the desired protein was concentrated to a volume of 4 ml by combined ultrafiltration and dialysis against 0 05 M phosphate buffer pH 7 0 made 0.5 M in respect of NaCl This solution (roughly 4 per cent) was applied to the top of a column filled with Sephadex G 200 equilibrated with the same buffer The filtration was started in the night by an electric switch watch at such a time that the desired fraction appeared in the effluent in the morning of the day that the centrifugation series of each component in different dilutions was to be run.

what has long been known as mucosal antibodies The special tendency of this protein to combine with other proteins would make it qualified for the rôle of an antiseptic paint It is also possible, however, that gamma A merely represents an ancestral form of immunoglobulin, initially exclusively associated with the lymphoid tissue from the connective structures sup porting the surfaces by which the organism makes contact with the antigens from the outside world

References

1 Ashbach N E Finstad J Sarnecki J C and Pollara B Fed Proceed 23 346 1964
2 Asofsky R and Thorbecke G J J Exptl Med 114 471, 1961
3 Burnet M F The Clonal Selection Theory of Acquired Immunity Cambridge University Press Cambridge 1959 p 86
4 Burrows W Elliott M E and Havens I J Infect Dis 81 261 1947
5 Chodirker W B and Tomasi T B Science 142 1080 1963
6 Crabbé P A Carbonara A O and Heremans J F Lab Invest 14 235 1965
7 Crabbe P A and Heremans J F Rev franç Etudes clin biol in the press
8 Crabbe P A and Heremans J F Gut in the press
9 Crabbé P A and Heremans J F unpublished
10 Davies A Lancet 2 1009 1922
11 Fireman P Vanuice W and Goodman H C J Exptl Med 117 603 1963
12 Freter R J Infect Dis 111 37 1962
13 Freter R and Gangarosa E J J Immunol 91 724 1963
14 Hanson L A Immunological studies of human milk with special reference to the immune globulins Orstadius Boktryckeri Goteborg 1961
15 Heremans J F Les globulines seriques du systeme gamma Arscia, Brussels and Masson Paris 1960
16 Heremans J F in J Šterzl (Ed) Molecular and Cellular Basis of Antibody Formation Czechosl Acad Sci Prague 1965 p 277
17 Heremans J F in S E Bjorkman (Ed) Proc 10th Congr Intern Soc Hematol Stockholm 1964 Vol 4 Munksgaard Copenhagen 1965 p 17
18 Heremans, J F Heremans M Th and Schultze H E Clin Chim Acta 4 96 1959
19 Heremans J F and Vaerman J P Nature 193 1091 1962
20 Hochwald, G M Jacobson E B and Thorbecke G J Fed Proceed 23 55? 1964
21 Ibayashi J Anzai T, Hood J E Hyde L and Carpenter C M Diseases Chest 44 514 1963
22 Ishizaka K Dennis E G and Hornbrook M J Allergy 35 143 1964
23 Ishizaka K Ishizaka T and Hornbrook M J Allergy 34 395 1963
24 Keimowitz R I J Lab Clin Med 63 54 1964
25 Masson P L Carbonara A O and Heremans J F Biochim Biophys Acta 107 485 1965
26 Masson P L and Heremans J F unpublished
27 Masson P L Heremans J F and Dive C unpublished
28 Masson P L Heremans J F and Prignot J Biochim Biophys Acta 111 466 1965
29 Remington J S Vosti K L Lietze A and Zimmerman A L J Clin Invest 43 1613 1964
30 Rockey J H Hanson L A Heremans J F and Kunkel H G J Lab Clin Med 63 205 1964
31 Straus L K Proc Soc Exptl Biol Med 106 617 1961
32 Tomasi T B Tan L M Solomon A and Prendergast R A J Exptl Med 121 101 1965
33 Tomasi T B and Zigelbaum S D J Clin Invest 42 1552 1963

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By Ulla Britt Hansson, C. B. Laurell and R. Bachmann

In 1965 Rowe and Fahey (9) described the characteristics of the new class of immunoglobulins IgD. One isolated IgD myeloma protein was found to have a sedimentation constant ($s_{20,w}$) of 7.0 against 6.6 for normal IgG. Of our series of 1300 sera with M components four were identified as IgD. Three of these samples were still large enough to allow isolation of the M component and estimation of the sedimentation constants. Their constants were lower than or the same as our constants for normal IgG. We therefore compared the sedimentation constants of some isolated M components of types IgG and IgD with those of some isolated individual normal immunoglobulins.

Material and methods

Frozen sera with M components of type IgD (number 510, 33 and 10 4) and of type IgG (number 165, 309, 8, 94 and 2?6) stored at −15° C for one to several years.

Fresh serum with M-components of type IgG (number 1311).

Fresh normal individual sera (number 1 to 8).

Paper electrophoresis was run in barbital buffer 0.05 M containing 2 mmol CaCl.

Isolation of M component (IgG and IgD) and of normal IgG. For the studies 0.25 to 0.30 g IgG (IgD) was used. One part serum was diluted with three parts 0.9% NaCl and ammonium sulphate was added to a final concentration of 1.84 M with stirring at room temperature. The pH was adjusted with ammonia to 7. After two hours at +4° C the precipitate was centrifuged down, washed, resuspended three times in ammonium sulphate (1.84 M) at pH 7 and +4° C and dissolved. The sample was dialysed first for at least 2 hours against running tap water and then overnight against 0.9% NaCl and finally for at least 5 hours against 0.02 M phosphate buffer pH 8.0. The material was fractionated on a DEAE column using a salt gradient with increasing ionic strength and decreasing pH (400 ml 0.02 M phosphate buffer pH 8.0 + 400 ml 0.2 M phosphate buffer pH 6). The eluate containing the desired protein was concentrated to a volume of 4 ml by combined ultrafiltration and dialysis against 0.05 M phosphate buffer pH ?.0 made 0.5 M in respect of NaCl. This solution (roughly 4 per cent) was applied to the top of a column filled with Sephadex G 200 equilibrated with the same buffer. The filtration was started in the night by an electric switch watch at such a time that the desired fraction appeared in the effluent in the morning of the day that the centrifugation series of each component in different dilutions was to be run.



Fig 1 Agar gel electrophoretic pattern of the ultracent rifuged M components and normal γ globulin fractions (For serial number see Table I) No 1311 represents preparation one The second appeared identical

Agar gel electrophoresis was run on 1 % agarose in 0 1 M barbital buffer pH 8 4

Ultracentrifugation was done at 59780 r p m in a Spinco Analytical Ultracentrifug Model L with Schlieren optics The temperature was 20° C and the buffer used was the same as that in the Sephadex column Each sample was run at three concentrations between 0 2 g per 100 ml to 1 g per 100 ml At each concentration five pictures 8 minute intervals were taken

Evaluation of the plates was done with two dimensional microcomparator (Leitz)

Calculation of sedimentation constants The differences (ln x_2—ln x_1)[1] were plotted (y axis) against the concentration (x axis) The straight line through the twelve points was calculated by the methods of least squares where the sum of squares of the vertical differences was reduced to a minimum The intersection of this line with the y axis gave the difference at a infinite dilution in buffer at 20° C The s value at infinite dilution in buffer at 20° C was calculated according to Schachman (11) and the s value in water at 20° C according to Jahnke and Scholtan (7) The standard error of a single determination was calculated and expressed as a percentage of the sedimentation constant

Immunologic identification M components 510 793 were classified by Dr J Fahey as I_gD M component 510 was purified and used to immunize rabbit The antiserum obtained was anti IgD specific after absorption Case 1074 was revealed with this antiserum Specific antisera against the immunoglobulin chains gamma kappa and lambda were available

[1] x_2 and x_1 are the distances from the centre of rotation to the maxima of peaks in cm at times t_2 and t_1

Results

The results of agarose gel electrophoresis of the purified immunoglobulins used are shown in Fig 1 The purified IgD M component No 1074 was contaminated with IgG The electrophoretic mobility of the IgD—M component (β—γ_1) agrees with the mobility given by Rowe and Fahey (10) for normal IgD globulins

The sedimentation constants calculated for the 3 IgD and the 6 IgG M components are compared with estimated constants of individual purified polyclonal IgG fractions (Table I) In the assessment of the reproducibility of the preparation the run and the evaluation the constant was estimated three times with the sample with serial No 3 and twice with sample 1311 The results obtained with the third preparation of No 3 showed that the apparatus was unaltered during the time used for the experiments Serial sample No 4 was also analysed twice but on the same day and with the same preparation to test the reproducibility of the run and the evaluation The standard errors expressed as per

Table I

Ultracentrifugal run No	Serial No	$s_{20,w}$ [1]	SD [2]	Globulin type	Paper electrophoretic mobility	Light chain type	Gm
56 57 58	510	6.54	0.9	M comp IgD	β−γ1	L	—
69 70 71	793	6.76	0.8		"	L	a—f—b—
126 127 128	1074	6.19	1.0		γ1	L	a—f—b—
109 110 111	165	6.81	0.4	M comp IgG	γ	K	a—f—b—
113 114 115	909	6.77	0.6	"	γ3	K	a—f—b—
116 117 118	8	6.46	0.6		γ1—γ2	K	a—f—b—
120 121 122	94	6.91	0.4		γ1	L	a—f+b—
123 124 125	226	6.72	0.4	"	β−γ1	K	a—f—b—
129 130 131	1311	6.82	0.5	"	γ1	K	a—f—b—
142 143 144	1311	6.78	1.0				
3 4 75	1	7.04	0.8	Normal IgG	γ1—γ3	—	a+f+b+
79 80 81	2	6.91	0.6			—	a—f+b+
6 77 78	3	6.88	1.0			—	a—f+b+
91 92 93	3	6.83	0.6			—	
151 152 153	3	6.90	0.5			—	
94 95 96	4	6.79	0.5	"		—	a+f+b+
99 100 101	4	6.78	0.3			—	
102 103 104	5	6.77	0.7			—	a+f+b+
139 140 141	6	6.[illegible]6	0.6			—	a+f+b+
145 146 147	[illegible]	6.81	0.6	"		—	a+f+b+
148 149 150	8	6.83	0.7		"	—	a+f+b+

[1] Sed. const. at *infinite dilution in water at 20* C

[2] Standard error expressed as percentage of the sedimentation constant

centage of the sedimentation constants varied between 0.3 and 1.0 (Table I). The values found for IgD M components varied between 6.19 and 6.76 and for IgG M components between 6.46 and 6.91. The IgD M components *represent the lowest values found*. The normal individual IgG preparations varied between 6.76 and 7.04. The light chain type of the M components are given in Table I. The three IgD M components had a light chain of type L. Of the six M components of type IgG five had a light chain of type K and one a light chain of type L. The Gm types were estimated by Dr R. Grubb on the individual normal and pathological IgG globulins and are given in Table I. All M components were Gm (a—f—b—) except No 94 that was Gm (a—f+b—). Of the normal immunoglobulins 6 were Gm (a+f+b+) and 2 were (a—f+b+).

Discussion

The molecular polymorphism of IgG is well known from *e.g.* variation in charge in Gm type and content of kappa or lambda chains if we disregard the difference in antibody specificity. The corresponding M compo-

nents are further known to vary e g in N-terminal amino acids and in peptide patterns on fingerprinting It is evident from the literature that the $s_{20,w}$-value found varied considerably among M components of 7 S-γ-globulin type But it is not possible to decide to what extent this depends on the measuring technique and the impurity of the preparation of the component since, in general, no data are available for the immuneglobulin type, degree of purity, ultracentrifugal and electrophoretic homogeneity The findings presented here indicate that freshly prepared M components of IgG and of IgD type vary in S rate outside the error of the analytical method Our IgD components gave s rates lower than, or equal to those of the IgG globulin (normal and pathological) in contrast to the single observation reported by Rowe and Fahey (9) Also the sedimentation constants for individual normal IgG varied outside the error of the analytical method It has to be elucidated whether this variation is correlated with any of the variations observed in the properties of the light or heavy chains of the immuneglobulins (1 2, 3, 4 5, 6 8) No link was found with the Gm system

Summary

The sedimentation constants for normal IgG globulins varied (6 76—7 04) outside the error of the analytical method Six IgG M components varied between 6 46 and 6 91 and 3 IgD M components gave 6 54, 6 76 and 6 19

Acknowledgement

This investigation was supported by grant from Swedish Medical Research Council (Project No 13 X 41 01

References

1 Fahey J L Contribution of γ globulin subunits to electrophoretic heterogenity identification of a distinctive group of 6, 6 S γ myeloma proteins Immunochemistry *1* 121 1964

2 Frangione B and Franklin E C Structural studies of human immunoglobulins Differences in the Fd fragments of the heavy chains of G myeloma proteins J exp Med *122* 1 1965

3 Franklin E C Structural studies of human 7 S γ globulin J exp Med *120* 691 1964

4 Grey H M and Kunkel H G H chain subgroups of myeloma proteins and normal 7 S γ globulin J exp Med *120* 253 1964

5 Grey H M and Mannik M Specificity of recombination of H and L chains from human γG myeloma proteins J exp Med *122* 619 1965

6 Harboe M Osterland C K Mannik M and Kunkel H G Genetic characters of human γ globulins in myeloma proteins J exp Med 116 719 1962

7 Janke K and Scholtan W Die Bluteiweisskorper in der Ultracentrifuge Georg Thieme Stuttgart 1960

8 Mannik M and Kunkel H G Classification of myeloma proteins Bence Jones proteins and macroglobulins into two groups on the basis of common antigenic characters J exp Med *116* 859 1962

9 Rowe D and Fahey J A new class of human immunoglobulins I A unique myeloma protein J exp Med *121* 171 1965

10 Rowe D and Fahey J A new class of human immunoglobulins II Normal serum IgD J exp Med *121* 185 1965

11 Schachman H K Ultracentrifugation in Biochemistry Academic Press New York 1959

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Ultracentrifugal Plasma Protein Pattern and Age in Healthy Men

By L. L. Böttiger, L. A. Carlson and S. Hedman

The pioneering work of Pedersen (8) in the 1940's made it possible to apply the analytical ultracentrifuge to the study of the composition of the serum proteins. One of the major advantages of this method is that it permits estimation of macroglobulins present in plasma. Waldenström was the first to introduce this technique into clinical medicine when he demonstrated the presence in serum of high amounts of macroglobulins in the syndrome later given his name (10, 11).

The interest in macroglobulins in Waldenström's disease and related disorders (cf. 4) and in connection with the rheumatoid factor and various antibodies has been steadily increasing. Nevertheless there are only few studies on the values for macroglobulins in healthy subjects (cf. 5). No material has been studied with regard to the possible influence of age. We therefore found it of value to establish normal levels in serum for macroglobulins in healthy individuals of various age.

Methods

Material

Sera from healthy men in different age groups were analyzed. The men had initially been randomly sampled from the population of Stockholm and had all taken part in a complete physical and laboratory health survey in 1954—55 (3) and again in 1958—59 (1). The criteria used to select the healthy men were described previously (1, 3).

In 1961 a group of men who were accepted as healthy in 1958—59 and whose health had not changed since, were called upon to participate in the present study.

The following laboratory tests were done: Hemoglobin, ESR and urinalysis for glucose and protein. The previously defined "normal" limits were used (1). Only two subjects were excluded, the reason being an ESR of 22 and 27 mm/hour respectively.

Analytical methods

Blood was withdrawn in the morning after fasting over night by venipuncture and allowed to clot at room temperature. Serum was separated off by centrifugation within 1 to 2 hours and its protein concentration determined with the Biuret method (13). The serum was diluted with buffer ($NaCl$ 0.17 M, Na_2HPO_4 0.03 M, NaH_2PO_4 0.02 M) to a protein concentration of 1 g per 100 ml. The ana

lysis were done in the Spinco Model L ultra centrifuge with the analytical rotor D and the 12 mm analytical cell at a speed of 59 780 r p m The runs were made at a rotor temperature of 20° C In all sera only three components were visible They have been called A G and M component (cf 5) in order of increasing sedimentation coefficient The relative amounts of the components were estimated by simple planimetry and the sedimentation coefficients of the components were calculated and adjusted to 20° C S_{20w} and the viscosity of water (5) No Johnston Ogston correction for superimposed gradients of slow components at faster boundaries was applied and the sedimentation rates obtained were not extrapolated to zero protein concentration (6)

Results

The concentration of total plasma proteins did not differ in the four age classes as seen in Table I The mean plasma protein concentration in the whole material was 7 8 g per 100 ml

Table I also shows that the percentage composition of the plasma proteins with regard to the three main ultracentrifugal fractions, A, G and M components, did not change with increasing age The mean value for the A component was 76 7 per cent and for the G and M components 18 3 and 5 0 per cent

As there were no differences in either the total amount or in the percentage composition of the plasma proteins all values were pooled and the *frequency distribution of the A, G* and M components studied Figure 1 shows the range of the values for these components and also indicates that there is no major deviation from a normal distribution

Table I Serum protein concentration, relative distribution and S_{20w} value of the A G and M components in the different age groups

Age group years		n	Serum proteins g/100 ml	A component per cent[1]	A component S_{20w}	G component per cent[1]	G component S_{20w}	M component per cent[1]	M component S_{20w}
30—39	M±SEM	18	7 8±0 1	77 5±0 9	4 0±0 0	17 7±0 8	6 8±0 1	4 7±0 5	17 0±0 1
	S		0 5	3 7	0 1	3 5	0 2	2 0	0 5
40—49	M±SEM	9	7 6±0 1	77 5±1 6	4 0±0 0	17 9±1 4	6 6±0 1	4 6±0 3	16 8±0 2
	S		0 4	4 8	0 1	4 3	0 2	0 8	0 5
50—64	M±SEM	15	7 9±0 1	75 1±1 3	4 0±0 0	19 1±1 1	6 6±0 1	5 8±0 5	16 8±0 1
	S		0 4	5 1	0 1	4 1	0 3	1 9	0 5
65—71	M±SEM	4	7 9±0 2	76 5±0 7	4 0±0 1	19 2±0 7	6 4±0 0[2]	4 3±0 2	16 5±0.3
	S		0 5	1 3	0 1	1 4	0 1	0 5	0 6
30—71	M±SEM	46	7 8±0 1	76 7±0 6	4 0±0 0[3]	18 3±0 5	6 6±0 0[3]	5 0±0 3	16 9±0 1[3]
	S		0 4	4 3	0 1	3 6	0 3	1 7	0 5

M=mean value SEM=Standard error of the mean S=Standard deviation n=number
S_{20w}=Sedimentation coefficient at the total protein concentration of 1 g per 100 ml adjusted to 20°C and the viscosity of water

1 =Per cent of total amount

2 =3 subjects

3 =45 subjects

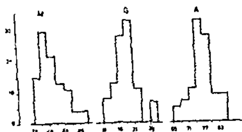


Figure 1 Frequency distribution of the three ultracentrifugal components in the whole material

The sedimentation *coefficient* for the three fractions did not vary with age (Table I) The mean value for the X component was 4 0 and for the G and M components 6 6 and 16 9 respectively

Discussion

The ratio albumin/globulin in serum determined with different methods such as precipitation and electrophoresis has been reported to decline with age in man (for review see 7)

In this material on the other hand the X component which contains the serum albumin did not change from 30 to 70 years The X component comprises however not only serum albumin but also other serum proteins such as the X component of Pedersen (8) but by far the major part is accounted for by the albumin It is not likely that increases in the minor non albumin constituents of the X component could have contributed to any major extent to our finding of a constant level of the X component by masking a decrease in the concentration of albumin The X component for instance which is lipoprotein and constitutes less than 10 per cent of the X component cannot have *increased* more than a few per cent from 30 to 70 years as evidenced by lipid and lipoprotein analysis of this material (1) It appears more likely that the differences are due to the composition of the materials studied It was indeed pointed out by Rafsky et al (9) that in their material of 31 "normal" old subjects chronic degenerative diseases may have been present Major diseases of that kind have most likely been excluded from our material of clinically healthy men In accordance with this Woodford Williams et al have recently reported that age has no apparent effect on serum protein levels in subjects considered disease free (14) They separated the serum proteins with electrophoresis and found that immobility and various disease processes and not age were responsible for observed decreases in the albumin/globulin ratio

Few materials are available on the ultracentrifugal composition of plasma proteins Values for 20 "normal" sera age not given analysed under conditions which separated the X component from the A component were given by Jahnke and Scholtan (5) as follows X component 5 6 A component 77 9 G component 13 9 and M component 2 6 per cent of total protein concentration Wallenius et al (12) who analysed lipid free sera found other values with lower concentration of the A component and higher of the G and M components He also analys

ed sera after zone electrophoretical separation and stressed the fact that the M component is composed of approximately the same amounts of alfa$_2$ and gamma globulins In four normal men (age 25—41, criteria for selection not given) Eriksen (2), analysing sera diluted 5—7 times with M/10 NaCl, found a mean value for the M component of 0 26 g/100 ml while our figure was 0 39 g/100 ml A direct comparison between these previous and the present results is not possible as the conditions of centrifugation (protein concentration, density, pH, temperature) were different in these studies, which may have a significant influence on the quantitative results

Summary

The serum protein concentration and composition analysed in the analytical ultracentrifuge has been studied in 46 clinically healthy and randomly selected men from 30 to 70 years The total serum protein concentration and the percentage amount of the A- G and M-components remained constant over this age span

References

1 Carlson L A Acta med scand *167* 377 1960
2 Eriksen N J Lab clin Med *51* 521 1958
3 Frisk A R Holmgren A Strom G., Werkö L Westman C & Viktorsson K E Nord Med *58* 1437, 1957
4 Heremans J F Heremans M Th Laurell A H I Laurell C B, Mårtensson L Sjöqmst J & Waldenström J Acta med scand suppl 367 1961
5 Jahnke K & Scholtan W Die Bluteiweisskörper in der Ultrazentrifuge G Thieme Stuttgart 1960
6 Johnston J P & Ogston A G Trans Faraday Soc. *42* 789 1946
7 Korenchevsky V Physiological and pathological ageing Karger Basel 1961
8 Pedersen K O Ultracentrifugal studies on serum and serum fractions Almqvist & Wiksell Upsala 1945
9 Rafsky H A Brill A A Stern K G & Corey H Amer J med Sci 224 522 1952
10 Waldenström J Acta med scand *117* 216 1944
11 Waldenstrom J Schweiz Med Wochenschr *78* *927* *1948*
12 Wallenius G Trautman R., Kunkel H G & Franklin E C J biol Chem 225 253 1957
13 Weichselbaum T E Amer J clin Path Tech Bull *10* 40 1946
14 Woodford Williams E Alvarez A S Webster D Landless B & Dixon M P Gerontologia *10* *86* 1964/65

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The Frequency of Pronounced Polyclonal Hypergammaglobulinaemia in a Random Population

By U. Axelsson and J. Hällén

On electrophoresis of serum an increased gamma globulin fraction is sometimes seen as a diffuse and sometimes as a more localized increase. The latter type is seen as a sharp peak in moving boundary electrophoresis and is a narrow band on paper electrophoresis. Referring to Burnet's clonal selection theory (2) Waldenström (13) called this type of increase monoclonal hypergammaglobulinaemia. This pattern is seen mostly in myelomatosis and macroglobulinaemia Waldenström but is also found in other conditions as described by Waldenström (12, 14, 15).

Diffuse or polyclonal hypergammaglobulinaemia is most pronounced and most common in systemic lupus and cirrhosis of the liver and is sometimes seen in rheumatoid arthritis, chronic sialoadenitis, thyroiditis and discoid lupus, i.e. conditions more or less closely related to each other and to the two first mentioned diseases (13). Protracted infections are often attended by moderate polyclonal hypergammaglobulinaemia. Such an increased gamma globulin fraction has also been found in apparently healthy persons (9). The heredity of polyclonal hypergammaglobulinaemia has been studied by Leonhardt (6).

From 1963 to 1965 a mass health control sponsored by the Swedish National Board of Health was carried out in a district of Sweden. At the suggestion of Professor Waldenström this investigation was extended to include paper electrophoresis of sera from the inhabitants in one part of the district, mainly to estimate the frequency of monoclonal hypergammaglobulinaemia in a random population. A paper dealing with the result of this investigation is in press (1). This paper is concerned with an estimate of the frequency of pronounced (≥2.0 g/100 ml) polyclonal hypergammaglobulinaemia in the population under discussion.

Material and methods

All persons above 25 years of age were invited to take part in the health control. During a 3 month period we obtained sera from

Table I Data concerning 12 cases with polyclonal hypergammaglobulinaemia

Case	Sex Age	Alb	γ	E S R mm/1 h	Waaler Rose	FIIA	A N F	Diagnosis
1 (4471)	F 43	5 6	2 2		128	2560	neg	No subjective symptoms
2 (75)	M 73	5 1	2 3	113	256	2560	neg	No subjective symptoms
3 (88)	F 52	5 4	2 0	40	128	2560	neg	Cardiosclerosis
4 (2482)	F 56	4 6	2 3	25	neg	neg	neg	Bronchial asthma cancer
5 (5422)	F 33	5 1	2 0	12	*64*	*2560*	*neg*	*Diffuse joint pain*
6 (105)	F 67	3 7	2 7	80	64	2560	neg	Rheumatoid arthritis
7 (5519)	F 60	5 2	2 3	105	128	2560	neg	Rheumatoid arthritis
8 (8300)	F 62	4 5	2 3	80	neg	neg	++	Rheumatoid arthritis
9 (449)	F 62	4 0	4 7	65	neg	80	++	Chronic hepatitis
10 (7108)	F 57	4 8	2 3	41	256	2560	neg	Cholangiolitis — *cirrhosis*
11 (6150)	F 40	4 4	3 4	128	neg	640	neg	Hyperglobulinaemic purpura
12 (6511)	F 46	5 1	2 9	92	32	neg	neg	Hyperglobulinaemic purpura

Alb γ albumin and gammaglobulin fractions (g/100 ml) on paper electrophoresis
Waaler Rose sensitized sheep cell test
FIIA *FII acryl particle fixation test*
A N F antinuclear factors

6995 persons *i e* 70% of the population above 25

All sera were studied by paper electrophoresis (4) The strips were inspected visually *for polyclonal hypergammaglobulinaemia* and the protein fractions of hyperglobulinaemic sera were studied quantitatively

At the Department of Clinical Bacteriology in Malmo (Head S Winblad M D) antinuclear factors (A N F) were demonstrated by the method used by Leonhardt (6) The heterophile absorbed sheep cell test (Waaler *Rose) and FII* acryl *particle fixation test* (FIIA) were done according to Winblad (17)

We tried to obtain as much pertinent information as possible about the subjects with pronounced hypergammaglobulinaemia Some *of them* were examined clinically because of abnormal findings in the blood chemistry at the screening of the mass health control Concerning the others *information* was *obtained* from hospitals or from the subjects themselves

Results

Polyclonal hypergammaglobulinaemia was suspected on visual inspection of the paper electrophoretic strips of 28 of *6995 sera studied The concentration* (g/100 ml) was less than 1 5 in 4 *1 5 or more in 12* and *2 0 or more in* 12 sera

Table I gives data about the 12 last mentioned cases One subject was a male aged 73 The mean age was 54 years

The highest gamma globulin value *found* was *4 7 g/100 ml Albumino*penia (<4 2 g/100 ml) was found in *two cases The E S R usually reached* high levels and was normal in only one case

The Waaler Rose test and/or the FIIA test usually both were clearly positive in 9 cases A N F were found in case 8 where rheumatoid factors were not found and in case 9 where only the FIIA test was positive

One woman (case 1) had always felt well The only man (*case 2*) in the

series was an old farmer who had been admitted to hospital because of mild anaemia and a high E.S.R. No signs of malignant disease had been found. Liver function had not been studied. One year later, at the time of the health control his general condition was good.

One woman (case 3) had been receiving medical treatment for several years for atrial fibrillation and cardiac insufficiency. She was known to have had a moderately high E.S.R. for years.

One woman (case 4) had had allergic rhinitis, asthma and drug allergy. She had also been operated upon for cancer of the vulva.

One young woman (case 5) had intermittent joint pains but no signs of arthritis. Three women (cases 6, 7 and 8) had rheumatoid arthritis. In one (case 7) of these the joint symptoms had been initiated by drug allergy and parotid swelling.

One woman (case 9) had had diabetes for 20 years. One year before our examination she had been treated in hospital, where jaundice, a high transaminase level and extremely high gamma globulin concentration (>6 g/100 ml) had been found. Her condition was conceived by us as chronic hepatitis. Five years before the health control one subject (case 10) had undergone appendectomy and a carcinoid had been diagnosed histologically. Three years later the E.S.R. had been high and a tumour had been suspected. Laparotomy had revealed nothing abnormal but histological examination of a liver biopsy specimen had shown cholangiolitis and moderately advanced cirrhosis.

Cases 11 and 12, aged 40 and 46, had purpura of the legs. The former had had the condition for two years and the legs were moderately discoloured. The latter had had this condition for 20 years and her legs were strongly discoloured up to the groins. The bleedings were more pronounced when she had been standing for a long time and during thunderstorms. Both patients otherwise felt well and were not at all incapacitated by their symptoms.

Discussion

In order to get a complete series in spite of the rough screening method only cases with pronounced (≥ 2.0 g/100 ml) hypergammaglobulinaemia were accepted in the present investigation.

The frequency was about 1 per 600 persons above 25 years of age. Hyperglobulinaemia of this level is often seen in association with various diseases such as systemic lupus and cirrhosis of the liver. It is very likely that some persons with such diseases did not take part in the health control because they were already under medical care. The frequency found must therefore be regarded as somewhat too low. For comparison it might be mentioned that two persons with monoclonal hypergammaglobulinaemia of 2.0 g/100 ml or more were found.

The mean age of the subjects (53 years) did not differ from that of the

primary series (51 years) The female dominance is in good agreement with the findings in series with such di seases as systemic lupus (8), non alcoholic liver cirrhosis (3) and rheu matoid arthritis (5)

The albumin concentration was decreased in only 2 subjects including one with symptoms of hepatic failure This patient also had the highest gamma globulin level These findings made us suspect chronic hepatitis or liver cirrhosis

The next highest gamma globulin levels were found in two women with purpura of the legs These cases were interpreted as having hyperglobulin- aemic purpura

The Waaler-Rose and the FIIA tests are positive in about 70 % and 80 % respectively in hospital patients with rheumatoid arthritis (5) In 72 cases with more or less clearcut systemic lupus, Leonhardt (6) found the results of both tests to be positive in 33 % Waldenstrom et al (16) found the Waaler Rose test to be positive in about 20 % of a liver cirrhosis series and especially in the cases with a high gamma globulin concentration Rheu matoid factors were found in most of our cases

Since the subjects described were in relatively good health further investi gations (liver function tests liver biopsy, L E cell test coagulation stu dies etc) were not considered well advised Our diagnoses were therefore based principally on the clinical his tory and the physical findings The conditions found were those expected in a series of hypergammaglobulin aemia Chronic liver disease, rheuma toid arthritis, drug allergy, parotid swelling and hyperglobulinaemic pur pura

Purpura hypergammaglobulin aemica first described by Walden strom (10, 11), is a relatively uncom mon finding especially in its primary form Among 72 cases reported Strauss (7) found 18 in which pur pura was described as an isolated phe nomenon The symptoms of hyperglo bulinaemic purpura are probably of ten so mild that medical advice is not sought This makes it difficult to esti mate the true frequency from hospital series In a random population we found two cases among seven thou sand persons above 25 years Cases are however, described with a gamma globulin concentration below 2 0 g/ 100 ml (7) so that we may have missed some cases

Summary

Sera from 6995 persons who coope rated in a mass health control were studied by paper electrophoresis Pro nounced (≥2 0 g/100 ml) polyclonal hypergammaglobulinaemia was found in 12 sera Nine of the 12 sera con tained rheumatic factors and anti nuclear factors were found in two There was only one man in the group of persons from which these sera were taken As expected most of the subjects had a history of such condi tions as rheumatoid arthritis liver

damage parotitis or drug allergy Two women had purpura hyperglobulin aemica

This study was supported by grants from Ernhold Lundstrom s Foundation, Malmo and Alfred Osterlund s Foundation Malmo

References

1 Axelsson U Bachmann R & Hallen J Frequency of pathological proteins (M components) in 6995 sera from an adult population Acta med scand In press 1966

2 Burnet M The clonal selection theory of acquired immunity Vanderbilt University Press Nashville 1959

3 Hallen J & Krook H Follow up studies on an unselected ten year material of 360 patients with liver cirrhosis in one community Acta med scand 1 3 4 9 1963

4 Laurell C B Laurell S & Skoog N Buffer composition in paper electrophoresis Clin Chem 2 99 1956

5 Lawrence J S Laine V A de Graaff R The epidemiology of rheumatoid arthritis in Northern Europe Proc roy Soc Med 54 454 1961

6 Leonhardt T Family studies in systemic lupus erythematosus Acta med scand suppl 416 1964

Strauss W G Purpura hyperglobulinaemica of Waldenstrom report of a case and review of the literature New Engl J Med 260 857 1959

8 Talbott J H & Ferrandis R M Collagen diseases p 2? Grune & Stratton New York 1956

9 Waldenström J Signs and causes of clinical hypergammaglobulinaemia The Svedberg 1884—1944 p 558 Almqvist & Wiksell Uppsala 1944

10 Waldenstrom, J Purpura hyperglobulinaemica Nord Med 23 1502 1944

11 Waldenstrom J Zwei interessante Syndrome mit Hyperglobulinamie (Purpura hyperglobulinaemica und Makroglobulinamie) Schweiz med Wschr 78 927 1948

12 Waldenstrom J Abnormal proteins in myeloma Advanc intern Med 5 398 1952

13 Waldenstrom J Studies on conditions associated with disturbed gamma globulin formation (gammopathies) Harvey Lect 56 211 1963 61

14 Waldenstrom J Clinical diagnosis and biochemical findings in material of 296 sera with M type narrow γ globulins Acta med scand suppl 367 110 1961

15 Waldenstrom, J The occurrence of benign essential monoclonal (M type) non macromolecular hyperglobulinemia and its differential diagnosis IV studies in the gammopathies Acta med scand 176 345 1964

16 Waldenstrom J Winblad S., Hallén, J., Liungman S & Persson B Hypergammaglobulinamie und Ausfall verschiedener serologischer Reaktionen bei alkoholischer und kryptogenetischer Leberzirrhose Acta hepato splenol (Stuttg) 11 347 1964

17 Winblad S Studies in laboratory estimation of rheumatoid arthritis serum factor 4 The role of gamma globulin and albumin for the acryl particles test for RAS factor Acta path microbiol scand 52 241 1961

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Monoclonal and Diclonal Gammapathies

By J W Imhof, R E Ballieux, N A J Mul and H Poen

— The immunoglobulins in normal human serum can be divided into four *classes* IgG, IgA, IgM and IgD Every immunoglobulin molecule is made up *of structural units known as* L (*for* light) and H (*for heavy*) *chains* The L chains are subunits which all four classes have in common The H-chains, however have a class specific structure and determine the immunochemical specificity of the class in *question (1)*

— Two structural types of L chain are known In accordance with the corresponding types of Bence Jones protein, they can be designated types I and II (2) Since every immunoglobulin molecule contains two identical L *chains these proteins* can be classified as type I or type II (3—6)

— *Heterogeneity of the H* chains has been established only for the IgG class (7—14) and is very likely on the basis of Harboe s (15) work *to be the* case also for the IgM class

— *In some* diseases the serum can contain an abnormal globulin fraction often referred to as "paraprotein or M component Such proteins have been mostly found in multiple myeloma and Waldenström's macroglobulinaemia, however, they can incidentally occur in many other diseases e g malignant tumours, reticuloses and collagen diseases (16—19) (table I)

— *On the* basis of the structural and immunological similarity to immunoglobulins or their structural units, the paraproteins[1] can be divided into classes and types

— In view of MacFarlane Burnet's "*clonal selection theory*" Waldenström suggested the designation "monoclonal gammapathy for paraproteinaemia (20) These cases are believed to be characterized by the existence of a single clone of immunologically competent cells such as lymphocytes *or plasma cells*, responsible for the production of one given type of protein (as manifested by the presence of a narrow banded hyperglobulinaemia or a paraprotein fraction in the elec

[1] The term "paraprotein" is used in this study only as a synonym of "M component"

Table I Classification of M-components

Immunoglobulin classes	Immunological types in each class	
	L chain	*H-chain*
IgG	type I and II ($\varkappa$ or λ)	type γ_{2a} γ_{2b} γ_{2c} γ_{2d} or γCr γCr* and γZu or type Ne We Vi and Ge
IgA	type I and II	—
IgM	type I and II	—
IgD	type I and II	—
Bence Jones proteins	type I and II	—
Franklin proteins* (Heavy chain or Franklin's disease)	—	type Cr and Zu

trophcrogram) One distinct abnormal protein fraction is found in the majority of patients with paraproteinaemia It is attractive to refer to these cases as monoclonal gammapathies

— In the course of the analysis of a large series of patients sera we observed besides the common form of single banded paraproteinaemia a number of patients with more than one serum paraprotein fraction This paper intends to describe the combinations found and to present arguments in favour of the designation diclonal paraproteinaemia with reference to some of these instances

Material and methods

Sera from 330 patients with paraproteinaemia were examined Agar electrophoresis was carried out according to Wieme (21) and immunoelectrophoresis according to Scheidegger (22)

For immunological typing of the paraprotein fractions antihuman serum of equine origin[2] specific anti IgG anti IgA and anti IgM serum, specific antitype I and type II L-chain serum all prepared in rabbits were used

Table II Classification and immunological typing of M components in cases of single banded paraproteinaemia

	L Chain type		Not typed	Total
	I	II		
IgG	71	45	44	160
IgM	35	18	63	116
IgA	31	21	23	75
B J	12	9	5	26
Total	149	93	135	377

Results

— A single banded paraproteinaemia was established in 377 patients The distribution over the various classes is shown in table II

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[2] Obtained from the Central Laboratory of the Bloodtransfusionservice of the Netherlands Red Cross — Amsterdam

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Monoclonal and Diclonal Gammapathies

By J. W. Imhof, R. E. Ballieux, N. A. J. Mul and H. Poen

— The immunoglobulins in normal human serum can be divided into four classes IgG, IgA, IgM and IgD. Every immunoglobulin molecule is made up *of structural units known as L-(for light)* and H (for heavy) chains. The L chains are subunits which all four classes have in common. The H chains, however, have a class specific structure and determine the immunochemical specificity of the class in *question (1)*.

— Two structural types of L chain are known. In accordance with the corresponding types of Bence Jones *protein they can be designated types* I and II (2). Since every immunoglobulin molecule contains two identical *L chains these proteins can be classi*fied as type I or type II (3—6).

— Heterogeneity of the H chains has been established only for the IgG class (7—14) and is very likely on the basis of Harboe's (15) work, to be the case also for the IgM class.

— *In some diseases the serum can* contain an abnormal globulin fraction often referred to as "paraprotein" or M component. Such proteins have been mostly found in multiple myeloma and Waldenstrom's macroglobulinaemia, however, they can incidentally occur in many other diseases, e.g. malignant tumours, reticuloses and *collagen diseases (16—19) (Table I)*.

— *On the basis of the structural and immunological similarity to im*munoglobulins or their structural units the paraproteins[1] can be divided into classes and types.

— In view of MacFarlane Burnet's '*clonal selection theory* Walden*strom suggested the designation "mo*noclonal gammapathy" for paraproteinaemia (20). These cases are believ*ed to be characterized by the existence of a single clone of immunologically* competent cells such as lymphocytes *or plasma cells responsible for the* production of one given type of protein (as manifested by the presence of a narrow banded hyperglobulinaemia or a paraprotein fraction in the elec

[1] The term "paraprotein" is used in this study only as a synonym of "M component".

teins The problem of the correlation between cellular structure and protein production has been approached by various techniques among which immunofluorescence microscopy and tissue cultures have made important contributions On the basis of these studies it seems likely that under pathological conditions one given clone of proliferating immunocytes produces one distinct pathological protein In these cases the "one clone/one protein theory" is acceptable as a working hypothesis at least if one *protein* is defined as one of the different types of immunoglobulins But Osserman (16) holds that the one clone/one protein hypothesis is disputable until proven On the basis of the demonstrated heterogeneity of the narrow banded protein components (24 25 26) the possibility of a single clone/multiple proteins situation and even that of a multiple clones/multiple proteins situation remains open However if at this moment we assume that the immunocyte dyscrasia reflects the neoplasia of an autonomic proliferation of a single cell clone resulting in the production of large amounts of a single class of immunoglobulins then we may refer to the single banded form of paraproteinaemia as monoclonal gammopathy (20)

— In some cases of paraproteinaemia the patient's serum contains two or several paraprotein components of two different classes of immunoglobulins In 1959 Curtain (27) described the co-occurrence of I_gM

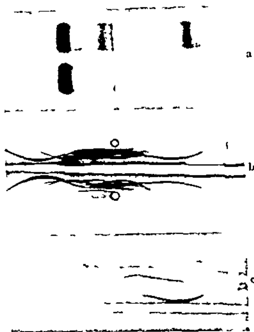


Figure 2 Analysis of the serum of case D In each slide the patients serum is on top and normal human serum as comparison on *the bottom*

a Agargel electrophoresis note the two M components

b Immuno electrophoresis the antiserum used was horse anti human serum The paraproteins are both of the G type

c Immuno electrophoresis the antiserum in the upper through is rabbit anti λ serum (anti type II L-chains) The antiserum in the lower through is rabbit anti κ serum (anti type I L chain) The faster moving M component is of the immunological type II the slower moving one is of type I

and I_gG paraproteins in the same patient

— We have previously reported on several patients in whom a dual paraproteinaemia was found (28 29) Bachmann and Laurell (30) also mentioned the occurrence of paraproteins

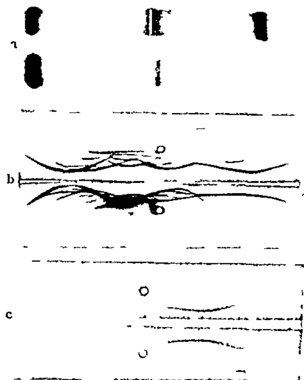


Figure 1 Analysis of the serum of case B In each slide the patients serum is on top and normal human serum as comparison on the bottom

a Agargel electrophoresis note the two M components

b Immuno electrophoresis the antiserum used was horse anti human serum The paraproteins are of the M type and of the G type respectively

c Immuno electrophoresis the antiserum used was rabbit anti $\varkappa$ serum (anti type I L chain)

Both M components are of the immunological type I The precipitation line of the IgM fraction is indicated by the dotted lines as the photographic reproduction is poor

— L chain typing was carried out (23) in 242 of these sera the results are presented in table II

— The electropherogram of 13 sera revealed more than one abnormal protein fraction Further analysis disclosed that co occurrence of a paraprotein fraction and a Bence Jones protein fraction of the same type existed in 7 cases Of these, 4 were of the IgG class (all being of the immunological type II), while the remaining 3 were of the IgA class (all abnormal protein fractions being of type I) In 5 cases, two paraprotein fractions of different class were detected in the same patient s serum The combinations found were

A $IgM^{I}+IgA^{I}$
B $IgG^{I}+IgM^{I}$
C $IgG^{I}+IgA^{I}$ (twice)
D $IgG^{I}+IgG^{II}$ [a]

In one case, a G myeloma protein of type I was detected in the serum whereas in the patient s serum and urine a type II Bence Jones protein could be demonstrated

E IgG^{I}+Bence Jones^{II}

— The results of the immuno electrophoretic studies in cases B D and E are presented in figures 1 2 and 3

Discussion

— In the majority of cases of paraproteinaemia the patient s serum contains a single abnormal protein fraction All these cases are characterized by an immunocyte dyscrasia and it is probable if not certain that the proliferating immunocytes (lymphocytes plasma cells) produce the parapro

[a] We thank Dr J W Stoop Department of Pediatrics State University Utrecht for making available to us the serum of patient D

proteinaemia whose urine contained Bence Jones proteins of type I as well as type II

— In one of our patients (E), we demonstrated the co-occurrence in the serum of an IgG paraprotein component with a type I L chain and a Bence Jones protein of type II

— The investigations made by Bernier and Cebra (33) tend to indicate that the different types of L chain are produced by different cells. Pernis and Chiappino (38) however reported on immunofluorescence experiments showing that in the white pulp of the spleen and in the germinal centres of the lymph nodes virtually all cells reacted with both types of L chain antisera. This might indicate that the immunocytes in the germinal centres produce both L chain types. At advancing maturation differentiation in protein production occurs, the mature immunocytes producing only one type of L chain and one class of immunoglobulins (Bernier and Putnam (39)).

The findings in our patient and those described by Engle and Nachman (37) might be explained by proliferation of two clones of mature immunocytes, each clone producing a different type of L chain; this would make the condition a diclonal gammapathy. An alternative possibility is that the paraproteins of different immunological types resulted from proliferation of a primitive clone of immunocytes still capable of producing both L chain types. A very recent communication of Pernis (40) however makes this assumption unlikely.

All things being equal the same applies to case D, in which the serum contained two paraprotein fractions both of the IgG class. L chain typing disclosed that the different IgG paraproteins were made up of different L-chains (IgG^{I} and IgG^{II}).

— Studies are now being carried out to establish whether these cases involve a diclonal paraproteinaemia or whether proliferation of a single clone of primitive immunocytes leads to the production of two different types of paraproteins.

Summary

— A review is presented of 390 cases of paraproteinaemia including 377 which involved a single paraprotein fraction (monoclonal gammapathy). The M components were classified as IgG in 160, IgA in 75, IgM in 116 and Bence Jones in 26 instances.

— In 13 cases the patient's serum contained two or several paraprotein fractions. In 7 cases the serum contained in addition to a G or A myeloma protein a Bence Jones protein of the same immunological type. In the remaining 6 cases the following combinations were found:

$IgM^{I} + IgA^{I}$
$IgG^{I} + IgM^{I}$
$IgG^{I} + IgA^{I}$ (twice)
IgG^{I} + Bence Jones^{II}
$IgG^{I} + IgG^{II}$

— The possibility that a diclonal gammapathy existed in these cases is discussed.

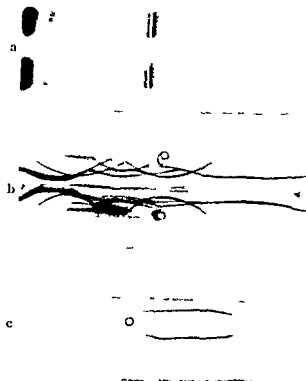


Figure 3 Analysis of the serum of case L. In each slide the patients serum is on top and normal human serum as comparison on the bottom

a Agargel electrophoresis note the two M components

b Immuno electrophoresis the antiserum used was horse anti human serum A G paraproteinfraction is present with a slower moving Bence Jones protein

c Immuno electrophoresis the antisera used were the same as in 2 c The faster moving M component is of the immunological type I the slower moving one is of type II

of different classes in the same patient

Kirstner and Norberg (31) reported on a patient with two paraprotein fractions of the IgA and IgG class respectively

— In the light of immunofluorescence investigations carried out by Curtain et al (27), by van Furth (32) and by Bernier and Cebra (33) it is highly probable that paraproteins of different classes (IgG, IgA and IgM) are produced by different cells It seems justified, therefore, to use the designation d i c l o n a l g a m m a p a t h y with reference to combinations of these paraproteinaemias In the patients' sera which we examined and presented under headings A, B and C, a diclonal paraproteinaemia existed in our opinion Evidence supporting this hypothesis is found in the fact that two different proliferating cell populations were found in the patients of groups A and B (i e plasma cells or myeloma cells and lymphatic cells in the bone marrow)

— A paraproteinaemia is not infrequently associated with Bence Jones proteinuria, while the serum contains a Bence Jones protein fraction besides the paraprotein component It can be assumed that in these cases the proliferating immunocytes produce an excess of free light chains In these cases which combine an abnormal serum protein component (IgG IgA or IgM) with Bence Jones proteinaemia or proteinuria the L chains of the serum component are nearly always of the same antigenic type (I or II) as the corresponding Bence Jones protein (34—36)

— In all these cases it is plausible that a single clone of neoplastically proliferating immunocytes produces an excess of L chains We found such a combination in 7 patients

— Engle and Nachmann (37) reported on a patient with IgG^{I} para

proteinaemia, whose urine contained Bence Jones proteins of type I as well as type II

— *In one of our patients (E)* we demonstrated the co-occurrence in the serum of an IgG paraprotein component with a type I L chain and a Bence Jones protein of type II

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Summary

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$IgG^{I} + IgG^{II}$

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References

1 I delman G M and Gally, J A — Proc Nat Acad Sc *51* 846 1964

2 Korngold L and Lipari R — Cancer *9* 262 1956

3 Franklin L C — Nature *195* 393 1962

4 Migita S and Putnam F W — J Exp Med *117* 81 1963

5 Mannik M and Kunkel, H G — J Exp Med *117* 213 1963

6 Fahey, J L — J Immunol *91* 438 1963

7 Lichter E A and Dray S — J Immunol *92* 91 1964

8 Terry W D and Fahey, J L — Fed Proc *23* 454 1964

9 Grey H M and Kunkel H G — J Exp Med *120* 253 1964

10 Ballieux R E Bernier G M Tominaga K *and Putnam* F W — Science *145* 168 1964

11 Takatsuki K and Osserman E F — Science *145* 499 1964

12 Ballieux R E Bernier G M Tominaga K and Putnam F W — Proc 2nd Meeting of the Fed Europ Biochem Soc Vienna 1965

13 Bernier G M Ballieux R E Tominaga K Easley C W and Putnam F W — 1966 in press

14 Poulik M D — Nature *204* 577 1964

15 Harboe M Deverill J and Godal H C — Scand J Haemat 2 *137* 1965

16 Osserman E F and Takatsuki K — Medicine *42* 357 1963

17 Danon F and Seligmann M — Proc Xth Congress Intern Soc Haemat Stockholm 1964

18 Imhof J W Ballieux R E Poen H *and* Mul N A J — *Prox* Xth *Congress* Intern Soc Haemat Stockholm 1964

19 Waldenstrom J — Acta Med Scand suppl *367* 110 1961

20 Waldenstrom J — Progress in Allergy *6* 320 1962

21 Wieme R J — Studies on agargel electrophoresis Editions Arscia Bruxelles (1959)

22 Scheidegger J J — Intern Arch Allergy Appl *Immunol 7 103 1955*

23 Mul N A J Ballieux R E Imhof J W and Kleinveld Fohrer A — Proc Xth Congress Intern Soc Haemat Stockholm 1964

24 Askonas B A — Biochem J *79* 33 *1961*

25 Fahey J L — Adv in Immunol 2 41 1962

26 Ballieux, R E — Structure analysis and classification of β_{2A} paraproteins Schotanus and *Jens* Utrecht (1963)

27 Curtain C A and O Dea *J* F — Austral Ann Med *8* 143 1959

28 Imhof J W and Ballieux R E — Proc IXth Congress Europ Soc Haemat Lisbo*a 1963*

29 Imhof J W and Ballieux R E — Folia Med Neerl 7 129 1964

30 Bachmann R — Acta med Scand 177 593 1965

31 Kirstner S and Norberg R — Proc Xth Congress Intern Soc Haemat Stockholm 1964

32 van Furth R — The formation of immunoglobulins by human tissues in vitro Luctor et Emergo Leiden 1964

33 Bernier G M and Cebra J J — J Immunol *95* 246 1965

34 Edelman G M and Gally J A — J Exp Med *116* 207 1962

35 Bernier G M Tominaga K Easly C W and Putnam F W — *Fed Proc 23* 558 1964

36 Gross and Epstein W — J Clin Invest *43* 83 1964

37 Engle R L and Nachman R L — Proc Xth Congress Intern Soc Haemat Stockholm 1964

38 Pernis B and Chiappino G — Immunology 7 500 1964

39 Bernier G M and Putnam F W — Progress in Hematology edit C V Moore and E B Brown p 160 Grune and Stratton New York 1964

40 Pernis B — Inform *Exchange group 5* no 44

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The Structure of Waldenstrom Macroglobulins [1]

By FRANK W PUTNAM MITSUO KOZURU and CAROLINE W EASLEY

Although the high molecular weight 19S globulins discovered in the serum of patients with macroglobulinemia by Waldenström (10) are among the largest of the well characterized proteins study of their primary structure is possible because they are polymers of subunits containing two kinds of polypeptide chains — heavy and light Since the light chains are structurally related to Bence Jones proteins whose amino acid sequence is being determined comparison of the tryptic peptide maps permits deductions as to the actual sequence of that portion of the light chains which is shared with Bence Jones proteins This study is facilitated by the relative homogeneity of individual Waldenstrom macroglobulins and the large amounts obtainable because of the hyperglobulinemia and the frequent use of plasmapheresis as a therapeutic procedure However a unique solution to the structural problem is precluded by the existence of two antigenic types (I and II) and the finding that macroglobulins from individual patients differ in structure The significance of structural study of Waldenström macroglobulins lies in the contribution to be made to the understanding of the variability of γ globulins and the biosynthesis of antibodies

For detailed structural study one must obtain well defined reproducible bits of the original molecules In the case of Waldenström macroglobulins reductive dissociation of the polymers yields monomer units with a molecular weight of about 160 000 — much too large for direct study Two further cleavage procedures have provided the necessary bits 1) partial enzymatic cleavage with papain and 2) separation of the light and heavy chains by *reduction and alkylation* In each case the cleavage products are digested with trypsin and the tryptic peptide maps prepared by standard procedures (7)

Papain cleavage of Waldenström macroglobulins does not yield two

[1] Supported by research grants (CA 02803 and CA 08[illegible]) of the National Cancer Institute National Institutes of Health Bethesda Maryland

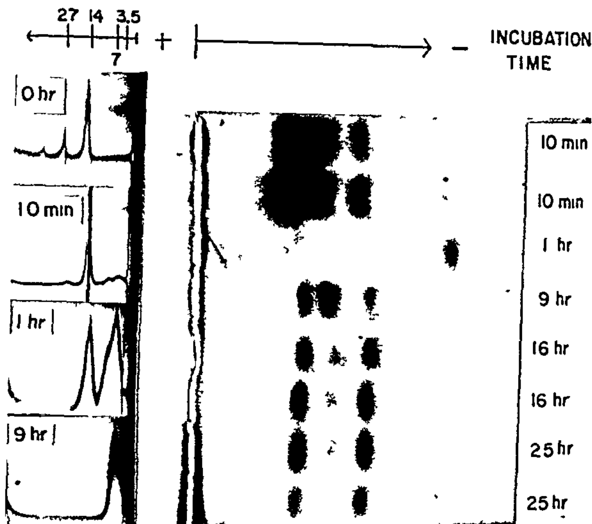


Fig 1 Composite illustration of the change in ultracentrifugal pattern and the starch gel electrophoretic pattern of a macroglobulin incubated with papain for increasing periods of time *Left* Ultracentrifugal pattern of the original macroglobulin (0 hr) and of the digests at 10 min 1 hr and 9 hr The direction of sedimentation and the approximate s_{20} are given at the top of the photograph The photographs are at different bar angles but were all taken at the same time after full speed was reached (16 min at 50 780 r p m) *Right* Starch gel electrophoresis at pH 5 5 at 250 volts for 6 hr of aliquots of the digest incubated for increasing times The direction of migration from the sample slot is given by the arrow In this experiment no protein moved towards the cathode and any intact macroglobulin remained in the starting slot

well defined parts of the molecule with different chemical and biological properties as in the case of 7S γ globulins However through study of the papain digest of one macroglobulin our laboratory first recognized the antigenic classification of myeloma globulins and macroglobulins into antigenic types I and II corresponding to the two antigenic types of Bence Jones proteins (6) Figure 1 illustrates the change in ultracentrifugal pattern and

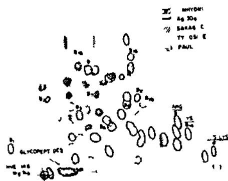


Fig 2 Tracing of the peptide pattern of the tryptic digest of an oxidized macroglobulin of antigenic type I (Na) as developed with ninhydrin and with the specific staining reagents identified in the figure legend Chromatography is in the vertical direction high voltage electrophoresis at pH 3 7 is in the horizontal direction Peptides designated Bi etc are present in most Bence Jones proteins of antigenic type I in normal human γ globulin and in myeloma globulins of antigenic type I Weak spots with ninhydrin are indicated by dotted lines

starch gel electrophoretic pattern of an antigenic type I macroglobulin (specimen Na) incubated with papain for increasing periods of time The polymer peaks disappear with the rapid formation of a 3 5 S component which represents all the ultracentrifugally detectable protein at the end of 4 hours Since the high molecular weight polymers are excluded from the starch gel the papain cleavage products are demonstrable at ten minutes Finally three electrophoretic bands remain By the Ouchterlony method and by starch gel immunoelectrophoresis these appeared antigenically identical By chromatography it was demonstrated that the whole papain digest consisted of low molecular weight dialyzable peptides a higher molecular weight glycopeptide and a 3 5 S fraction that gave the three banded starch gel electrophoretic pattern illustrated in Fig 1 From antigenic studies and from the peptide map illustrated later we concluded that sulfhydryl activated papain dissociates the polymer into subunits degrades a part of the heavy chain to low molecular weight peptides and leaves a series of closely related 3 5 S fragments that represent the light chain plus part of the heavy chain

Historically the most significant result obtained from papain digestion of macroglobulins was the recognition of the antigenic classification of all human immunoglobulins into two antigenic types Antiserum prepared in rabbits against the papain digest of the Na macroglobulin unexpectedly proved to be the best reagent for antigenic typing of Bence Jones proteins The antiserum reacted strongly with all type I Bence Jones proteins but not at all with type II This suggested to us that Waldenstrom macroglobulins were either antigenic type I or II Further study revealed that myeloma globulins likewise could be divided into antigenic type I or II (6) This led to the classification of all pathological human serum γ globulins into the same two antigenic types as Bence Jones proteins a conclusion independently arrived at in several laboratories (3 5)

The antigenic similarity of the papain fragment of the antigenic type I macroglobulin Na to type I Bence Jones proteins prompted a study of

their structural relationship by the "fingerprint" method Figure 2 illustrates the tryptic peptide map of the performic acid oxidized macroglobulin Na Whereas there are about 120 moles of arginine and lysine per monomer of 160,000 molecular weight, only about half as many spots are discernible in the figure This suggests the presence of several subunits within the monomer, namely, the heavy and light chains

By the use of multiple staining procedures (2) and mixtures of the tryptic digests of this macroglobulin and various type I Bence Jones proteins, fifteen peptides could be identified as present in the latter and thus representing the light chains of the macroglobulin These peptides are identified in Fig 2 by the designations B_1 B_2 etc Of the peptides so identified in Fig 2, the complete amino acid sequence has been reported for the following peptides that have been isolated from a type I Bence Jones protein B_1 B_3 B_6 B_7, B_8 B_9 B_{10}, B_{11} B_{13} B_{14} B_{15} B_{16} and B_{18} (7 9)

B_3 is the amino terminal octadeca-*peptide of many type I light chains* it begins with aspartic acid, has the only methionine residue in the light chain, and its sequence is known (8) B_8 and B_{16} are in the variable portion of the light chain just preceding the COOH terminal *half the latter* which seems to be almost invariant in type I light chains contains the peptides B_{18} B_{11} B_{10} B_6 B_{15} B_7, B_9 and B_1 (9) Thus the amino acid sequence of the light chain of the macroglobulin Na whose peptide map is *illustrated* in Fig 2, is very similar to that of a type I Bence Jones protein for which the amino acid sequence is almost completely known In general, the light chains of type I macroglobulins are very similar in primary structure to type I Bence-Jones proteins However, since the latter differ individually in sequence in their amino terminal halves, but are almost identical in their carboxyl terminal halves (9), it was necessary to compare the peptide maps of *the light* chains of a number of type I macroglobulins To do this separation into the light and heavy chains was required

The light and heavy chains of four type I macroglobulins and one type II macroglobulin were dissociated by reduction and alkylation by the method of Fleischman *et al* (4) followed by separation on a G 100 Sephadex column The purity of the fractionated chains was established by starch gel electrophoresis, and by immunodiffusion with antiserum specific for the heavy chains of γG and γA globulins and for type I and II light chains, as well as the intact globulins of the three major antigenic classes (γG γA and γM) On the basis of optical absorbancy at 280mμ the light chains constituted about 25 % of the macroglobulin molecule and the heavy chains about 75 % From amino acid *analysis of four* macroglobulins of type I it was evident that the heavy and light chains differ markedly in composition but are complementary with respect to the whole macroglobulins In every case where the heavy *and light chains* differed significantly



Fig 3 Peptide maps of a type I macroglobulin (Di) in the center and of the heavy chain (left) and the light chain (right) The peptides were developed with ninhydrin and the circled peptides were positive for arginine In the central map the arrows indicate peptides due to the light chain in the map for the light chain the peptides denoted B_1 etc., correspond to similarly designated peptides in Fig 2 The conditions are as in Fig 2 except that the macroglobulin and its polypeptide chains were reduced and alkylated This changes the position of sulfur containing peptides such as B_1 and tryptophan containing peptides such as B_{10}.

in amino acid composition the analytical values for the whole macroglobulin were intermediate

The complementarity of the heavy and light chains is illustrated by the peptide maps of Fig 3 The center photograph is the peptide map of the soluble tryptic peptides of the 19S macroglobulin from a patient (Di) with Waldenstrom macroglobulinemia The left and right panels represent respectively the heavy (H) and light (L) chains It should be noted that the map of the intact macroglobulin is almost a faithful composite of the maps of its constituent heavy and light chains For example the glycopeptides (the two dark spots on the abscissa near the origin) are present only in the heavy chain and the whole macroglobulin whereas certain peptides that are faint in the map of the whole macroglobulin become strong in the maps for the individual chains One example is B_4 a peptide containing 20 amino acids for which the order has been determined (positions 148—167 in the sequence given by Titani *et al* (9)) In principle the sequence of type I macroglobulins will be about one third determined when the sequence of type I Bence Jones proteins is completed

Comparative study of the peptide maps of the light chains of four type I macroglobulins confirmed their structural relationship to Bence Jones proteins as evidenced by the sharing of the B peptides The comparative study likewise supported the concept that the light chains of macroglobulins from individual patients differ from each other in a number of peptides though possessing many peptides in common As in the Bence Jones proteins structural differences seemed to be localized in the amino terminal portions of the chains For

example, in two macroglobulin light chains the amino terminal peptide B_3 was present, this begins with aspartic acid and contains methionine (8) However, the light chains from two *other macroglobulins both lacked the* amino terminal peptide B_3 One chain began with aspartic acid but lacked methionine, the other light chain began with glutamic acid and contained methionine Thus, there are at least three ways in which type I macroglobulin light chains can begin However, all light chains and Bence-Jones proteins of type I appear to end in the same way because they all contain the carboxyl terminal peptide B_1 (Gly Glu CySH) The type II light chains differ greatly in structure from the type I light chains but seem to be made up on a similar principle

Although the structural study of macroglobulin light chains is well advanced, only comparative study of the heavy chains by the peptide map method has been undertaken As in the case of γA heavy chains (1) the heavy chains of Waldenstrom macroglobulins share many peptides but differ in many *others It appears as if a portion* of the heavy chain is shared by all macroglobulins and another portion *differs for the individual patient* Future studies are needed to define the role of the heavy and light chains, to establish the location and function of the interchain and intermolecular di*sulfide* bonds, and to elucidate the structure of the carbohydrate moiety Ultimately, the structural study of Waldenstrom macroglobulins should contribute greatly to knowledge of the structure and biosynthesis of antibodies

References

1 *Bernier G M, Tominaga K* Easley C W and Putnam F W Biochemistry *4* 2072 1965

2 Easley C W Biochem Biophys Acta *107* 386 1965

3 Fahey J L J Immun *91* 483 1963

4 *Fleischman J B Porter R R* and Press E M Biochem J *88* 220 1963

5 Mannik M and Kunkel H G J Exp Med *116* 859 1962

6 Migita S and Putnam F W J Exp Med *117* 81 1963

7 Putnam, F W and *Easley C W* J Biol Chem *240* 1626 1965

8 Titani K and Putnam F W Science *147* 1304 1965

9 Titani K Whitley E Jr Avogardo L and Putnam F W Science *149* 1090 1965

10 Waldenstrom J Acta Med Scand *117* 216 1944

From the Arthritis Unit Department of Medicine University of Minnesota Hospitals Minneapolis, Minnesota

Studies on the Macroglobulins of Human Serum II Heterogeneity of Antigenic Determinants Among M-Components in Waldenstrom's Macroglobulinemia[1]

By Frank A Wollheim and Ralph C Williams Jr.

Starting with the observation by Oudin (15) on allotypy among rabbit gamma globulins an increasing number of serum proteins have been shown to exhibit genetically determined subgroups The Gm groups (9) are well known in this regard Recently subgroups of human γ chains (3) have been described independently in the laboratories of Kunkel (7) Fahey (18) and Putnam (1) and these have shown correlation to the previously mentioned Gm groups (12) The possibility of subgroups of the other classes of immunoglobulins is attracting increasing attention IgA is known to show allotypes in mice (10) Seligmann (16) has reported familial occurrence of macro M components and further more obtained antisera that would react with some but not with other such M components Some insight into the heterogeneity of macroglobulin antigens was presented by Deutsch and MacKenzie (4) By immunizing monkeys with IgM Rh antibodies from individual sera anti IgM antisera were produced which reacted with a varying number of a panel of other 19 S Rh antibodies Similarly our own experiments utilizing heterophile sera from patients with infectious mononucleosis showed marked variation in patterns of inhibition by different anti IgM antisera prepared in rabbits (20) The present investigation was undertaken in an attempt to study the antigenic structure of 8 monoclonal IgM proteins and the heavy and light chains prepared from them Utilizing rabbit antisera to individual macroglobulin M components evidence was obtained not only for the well known homologous individual specificity (8) but for varying degrees of cross reactivity with heterologous chains thus indicating the existence of subgroups among both heavy and light chains In addition it was a constant finding that the native IgM molecule always contained antigenic determinants in ex

[1] Aided in part by grants from the Minnesota Arthritis Foundation and USPHS # AM 0 3

cess of both isolated chains and mixtures of heavy and light chains, in other words, the native whole structure or undissociated macroglobulin contained antigens not found in its parts or chains produced by reduction and alkylation

Materials and Methods

All eight sera utilized in this study showed a well defined monoclonal spike on paper and agar electrophoresis The M component was isolated and shown to be immunologically pure IgM by immunoelectrophoresis In two instances (Ka and Ro) the whole sera gave good euglobulin precipitates on dilution with 9 parts of water and this was used as the first purification step The other six M components were isolated by starch block electrophoresis (11) Final purification was achieved by chromatography on DEAE Sephadex G 50 using stepwise elution with 0 15 M PO_4 pH 6 8 and 0 3 M PO_4 pH 6 8 The pure IgM occurred in the last step Rabbit antisera were prepared by immunizing with isolated Waldenström s IgM proteins in complete Freund s adjuvant followed in some instances by 3 intravenous injections of alum precipitated antigen Macroglobulin μ and L chains were prepared in the following way adapted after Fleischman Pain and Porter (5) 10 mg of the isolated macroglobulin in 2—4 cc 0 15 M NaCl were mixed with an equal volume of 0 2 M Tris buffer pH 8 0 2 M EDTA was added to give a final concentration of 0 002 M The mixture was degassed and put under an atmosphere of nitrogen 2 Mercaptoethanol was then added to a concentration of 0 1 M and the mixture left at room temperature for 60—90 minutes It was then cooled in ice water and a slight molar excess of iodoacetamide was added After another 60—90 minutes at 0° C dialysis vs 0 5 M propionic acid was started This was continued for 16—20 hours with one change of acid after 3 hours The reduction mixture was then concentrated by negative pressure dialysis to 1 cc and applied to a Sephadex G 100 column measuring 1 2 × 140 cm equilibrated with propionic acid Figure 1 shows a typical elution pattern The peaks were pooled dialyzed against 0 1 M acetate buffer pH 5 5 concentrated to 1—3 mg protein per cc and stored at —20° C. Ouchterlony double diffusion experiments were performed in 2 % agar at 4° C and the plates were observed for at least 1 week. Tanned sheep cells coated with IgM heavy chains were prepared after the method of Boyden (2) Inhibition experiments were performed by incubating 0 1 cc aliquots of various rabbit anti IgM antisera with 0 1 cc IgM preparations in serial dilutions for 30 minutes at room temperature before adding 1 drop of the 1 % tanned cell suspension The tubes were read after overnight standing at 4° C

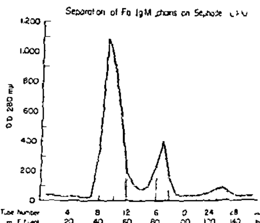


Figure 1 Typical gel filtration chromatogram obtained after reduction alkylation and dialysis of 10 mg pure macro M component. A 1 2 × 140 cm column with Sephadex G 100 was equilibrated and eluted with 0 5 N propionic acid Peak 1 (6—7 mg protein) contained heavy chains with no or only trace reactions with anti light chain antisera Peak 2 contained light chains peak 3 contained no identifiable antigenic determinants

Results

Figure 2 shows a typical pattern obtained by double diffusion of Ka IgM and chain preparations of this protein

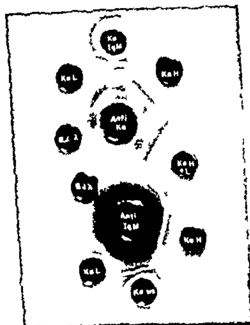


Figure 2 Ouchterlony experiment on Ka. Anti Ka = rabbit anti Ka IgM absorbed with cord serum. Anti IgM = rabbit antiserum to polyclonal IgM absorbed with cord serum. Ka WS = Ka whole serum. Ka IgM = isolated M component. Ka H = Ka heavy chains. Ka L = Ka light chains. Ka H+L = mixture of H and L. Bence Jones λ = isolated Bence Jones protein of group λ. The Ka IgM was typed as λ.



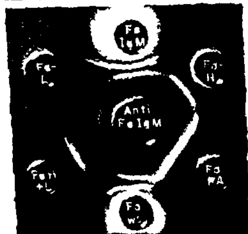


Figure 3 Ouchterlony experiment on Fa. Anti Fa IgM = anti Fa macro M component absorbed with cord serum. Fa WS = Fa whole serum. Fa IgM = isolated M component. Fa H = heavy chains. Fa L = light chains. Fa RA = reduced alkylated Fa IgM. Fa H+L = chains mixed after initial separation.

with an homologous and one heterologous antiserum both absorbed with human cord serum. It can be seen that the intact IgM spurs over both its heavy and light chains and this was also true in relation to unseparated mixture of alkylated chains. It can also be seen that the homologous antiserum reacts with the L chains despite the fact that kappa and lambda determinants were absorbed out with cord serum. Similar patterns were obtained with all examined IgM preparations and a variety of anti IgM antisera. Figure 3 shows the reactions between anti Fa and Fa IgM and Fa chains. It can be seen that the unreduced IgM already contains some heavy chain like material. The same situation was also observed with *Ker IgM*.

Having thus established the fact *that whole IgM has antigenic determi*

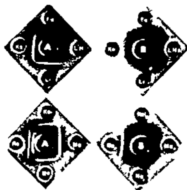


Figure 4 Ouchterlony experiment with 8 different IgM heavy chain preparations A = anti Ro IgM absorbed with cord serum B = anti Ro IgM absorbed with cord serum and Kei whole serum

Table I Precipitin Reactions of IgM heavy chains (μ) in Ouchterlony double diffusion with anti IgM Ro absorbed with cord serum, or with cord serum and macroglobulinemic serum, and with some other cord serum absorbed antimonoclonal IgM antisera

Antiserum	IgM heavy chains 1 0 mg cc							
	Fa	L H	Li	Ka	Kei	Ma	No	Ro
Anti Ro abs cord	+	+	+	+	+	+	+	++
Anti Ro abs L H	—	±	—	+	+	+	+	+
Anti Ro abs Li	—	—	—	+	—	±	+	+
Anti Ro abs Kei	—	—	—	+	—	+	+	+
Anti Ro abs Ka	—	—	—	—	—	—	—	+
Anti Ro abs Fa	—	—	—	—	—	—	—	+
Anti Ro abs Ma	—	—	—	—	—	—	—	+
Anti Ro abs No	—	—	—	—	—	—	—	+
Anti Ka abs cord	—	—	+	++	—	+	+	++
Anti Fa abs cord	+	—	+	+	—	+	+	++
Anti Kei abs cord	+	—	+	+	+	+	+	+

minants lost during reduction, attempts were made to detect antigenic subgroups In general specific anti IgM antisera prepared against monoclonal Waldenstrom's macroglobulins and absorbed with cord serum reacted with all 8 purified IgM preparations in addition to showing individual specificity for the homologous IgM proteins These results are similar to those obtained by Grey, et al (8) with M components of the IgG, IgA and IgM subclasses of immunoglobulins Absorption of such antisera with small amounts of heterologous whole macroglobulinemia serum resulted in loss of the precipitin reaction with all 8 intact IgM preparations However such absorbed antisera were still able to react with heavy chains from some but not from other IgM globulins Figure 4 shows the result of a typical experiment Table I summarizes the results obtained thus far with the 8 heavy chains of the panel Whereas anti Ro absorbed with cord serum only reacted with all 8 μ chains of the panel the similarly absorbed anti Ka failed to precipitate with Fa L H and Kei μ chains the anti Fa reacted with all except L H and Kei and the anti Kei with all except L H Absorption of anti Ro with either Li or Kei whole serum resulted in identical antisera reacting with only Ka, Ma No and Ro Absorption with Ka Fa, Ma and No all give antisera only reacting with the homologous Ro μ chains thus leaving only antibodies to the individual μ chain determinant of Ro It is evident from these experiments that Ka Ma No and Ro form a closely related subgroup The existence of other subgroups is suggested by the reactions with anti Fa and anti Kei

Table II Inhibition of sheep erythrocytes, tanned with L'H heavy chains Agglutinator 1 1500 dilution of anti Kei IgM Agglutinator and inhibitor incubated for 30 mins before addition of test cells[1]

Inhibitor	Concentration mg/cc .5	25	125	063	032	016	008	004	002	001	0005	00025	00013	00006
Fa IgM	NT	—	—	—	—	—	—	—	1	1	1—2	2	2	3
L'H IgM	—	—	—	—	1	1	1—2	2	2	3	3	3	3	3
Li IgM	—	—	—	—	1	2	2	3	3	3	3	3	3	3
Kei IgM	—	—	—	—	—	—	—	—	1	1	1—2	3	3	3
Ma IgM	—	—	—	—	—	1	2	2	3	3	3	3	3	3

[1] Reactions were read after overnight incubation in the cold room in a one to three scale

but our data with these antisera are as yet incomplete

The light chains reacted in general only with their homologous antiserum but an exception from this rule was anti Ro which reacted with light chains from Kei Ma and weakly with Fa and Li (Figure 5)

Table II shows the result of an haemagglutination inhibition experiment with L H heavy chains tanned on sheep cells anti Fa and whole IgM preparations as inhibitors It can be seen that Fa and Kei were most potent inhibitors Ma was intermediate and Li and L H inhibited most weakly It was thus possible to show serological differences among M components which could be confirmed in agar diffusion experiments with the same antiserum Technical difficulties have thus far not permitted us to expand experiments of this type

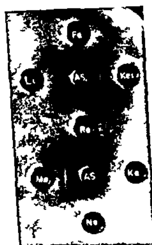


Figure 5 Ouchterlony experiment with 7 different IgM light chain preparations AS = anti Ro IgM absorbed with cord serum.

Discussion and Conclusions

In the present study reduction and alkylation of human IgM globulins gave rise to heavy and light chains in about the same ratio as is the case with IgG This may indicate a similar structure of these two proteins However Ouchterlony antigenic analysis has shown that major precipitating antigenic determinants are lost upon such reduction implying that intact disulfide bonds (between heavy and light chains) or relatively labile intra chain disulfides are essential for stabilizing or shaping much of the specific IgM antigenic site The conforma

tion of the native IgM molecule thus may be of paramount importance for antigenicity, much in the same fashion as has been shown to be the case with ribonuclease (14) It has been shown, that reduction of three disulfide bonds in the single chain of this polypeptide without any loss of amino acids completely destroys the reaction with specific antisera Similar observations have recently been made by Gerstein, et al (6) with respect to pepsinogen

The fact that all individual IgM preparations in our experience reacted with all strong antisera to intact monoclonal IgM, as well as that absorption with one IgM abolished reactivity with all other intact IgM preparations indicates a high degree of cross reactivity among these M components On the other hand the heavy chains derived from them show marked differences in antigenic specificity, and seem to fall into subgroups that are hidden in the intact IgM molecule This H chain subgrouping appeared to be a relatively buried antigenic determinant since it was brought out by anti-IgM antisera absorbed with whole macroglobulin sera

The sensitive serological method of hemagglutination inhibition is probably more suited to detect differences of the antigenic structure of whole IgM, as indicated in Table II This view is also supported by observations of Deutsch and MacKenzie (4) on monkey antisera to IgM Rh antibodies and by the present authors in inhibition of IgM heterophile antibodies with antisera to monoclonal IgM (20) MacKenzie and Deutsch (13) have in their recent paper further extended their observations on inhibition of monkey antisera to individual anti IgM Rh antibodies and Waldenström's macroglobulins They found the same phenomenon of differences in inhibition of individual antibodies from different sera to be true also of anti A and anti-B mercaptoethanol sensitive blood group antibodies, but not on the whole of cold agglutinins Whereas the monkey antisera used by these authors thus behaved very similarly to our rabbit antisera in inhibition of serologically active IgM antibodies they failed apparently to recognize both the hidden individual light chain determinants (19) and the H chain determinants described in this paper This failure to precipitate chains could be due to either the technique used for immunization or to the animal species used It is also surprising to note absence of individual specificity of their anti Waldenström antisera for the homologous whole IgM

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Summary

Human monoclonal IgM was reduced in 0 1 M 2 mercaptoethanol and heavy

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Bibliography

1 Balieux R E Bernier G M Tominaga K and Putnam F W Science 145 168, 1964

2 Bowden S A J Exp Med 93 107 1951

3 Bull W H O 30 447 1964

4 Deutsch H F and Mackenzie M R Nature 201 87 1964

5 Fleischman J B Pain R H and Porter R R Arch Biochem Biophys Suppl 1 174 1962

6 Gerstein J F Levine L and Van Vunakis H Immunochemistry 1 3 1964

7 Grey H M and Kunkel H G J Exp Med 120 253 1964

8 Grey H M Mannik M and Kunkel H G J Exp Med 121 561 1965

9 Grubb R and Laurell A B Acta Path Microbiol Scand 39 390 1956

10 Herzenberg L A Cold spring harbor symp Quant Biol 1964 In press

11 Kunkel H G Meth Biochem Anal 1 141 1954

12 Kunkel H G Allen J C, Grey H M Martensson L and Grubb R Nature 203 413 1964

13 Mackenzie M R and Deutsch H F J Immunol 95 87 1965

14 Mills J A and Haber E Fed Proc 21 A 31 1962

15 Oudin J J Exp Med 112 107 and 125 1960

16 Seligmann M and Badin J Rev Franc Etudes Clin. Biol 7 1107 1962

17 Solomon A Litwin S D and Kunkel H G Fed Proc 24 201 1965

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The fact that all individual IgM preparations in our experience reacted with all strong antisera to intact monoclonal IgM, as well as that absorption with one IgM abolished reactivity with all other intact IgM preparations indicates a high degree of cross reactivity among these M-components. On the other hand the heavy chains derived from them show marked differences in antigenic specificity, and seem to fall into subgroups that are hidden in the intact IgM molecule. This H-chain subgrouping appeared to be a relatively buried antigenic determinant since it was brought out by anti-IgM antisera absorbed with whole macroglobulin sera.

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Bibliography

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19 Williams R C Jr Proc Nat Acad Sci 52 60 1964
20 Wollheim F A and Williams R C Jr N Eng J Med in press 1965

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A New Immunological Test for Monoclonal Macroglobulinemia — A Preliminary Report

By Leonhard Korngold

In 1944 Waldenström (3) described the first cases of macroglobulinemia — a syndrome characterized by a tremendous increase in a γ globulin with a sedimentation constant of 19 S This protein, which has a molecular weight of 1,000,000 is now known to be a member of the immunoglobulins and is designated as IgM or γM In 1962 Waldenstrom suggested that conditions giving rise to unusual concentrations of the immunoglobulins be called gammapathies (4) and that they be subdivided into polyclonal or monoclonal gammapathies according to their electrophoretic pattern — i e whether the immunoglobulin region was diffuse (polyclonal) or characterized by a narrow spike (monoclonal)

During the last few years it has been established that all immunoglobulins can be subdivided into two antigenic types, now designated K and L, and that these antigenic configurations reside in the light chains, which are common to all immunoglobulins (1)

Our laboratory has been interested in devising immunologic tests for the gammapathies based on the fact that in the polyclonal gammapathies both antigenic types become proportionally elevated whereas, in the monoclonal gammapathies one of these types becomes predominant (2)

Moreover, we wanted to do this with antisera that could distinguish between the γG γA and γM gammapathies without having to isolate the suspected immunoglobulins This has been accomplished without much difficulty for the γG and γA gammapathies The γM gammapathies on the other hand presented problems, which seemed unsurmountable for theoretical reasons Since the detection of the K and L types of the γM proteins depended on the presence of antibody against either the kappa or lambda chains the γM globulins always had to be isolated in re

The author s work was supported by Research Grants C 4798 and C 4799 from the National Cancer Institute National Institute of Health Public Health Service

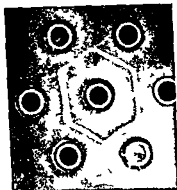


Figure 1 Ouchterlony gel diffusion plate Center anti γML serum absorbed with γG globulin Top left and bottom right normal serum (1 2) The other reservoirs contain sera (1 10) from patients with monoclonal gamma M pathies of type K

latively pure form otherwise the smaller (and more rapidly diffusing) γG proteins would interfere with the typing

The present paper will discuss the preparation of anti γM antisera that lack antibodies against the light chains but which nevertheless can be used for typing the γM globulins

Materials and Methods

γM globulins — γM globulins were isolated from the sera of patients with macroglobulinemia of Waldenstrom Some of these proteins were cryoglobulins and were purified by repeated precipitation in the cold and solubilization at 3 °C The other γM preparations were obtained by preparative starch block electrophoresis

Immunization — Rabbits were inocculated with the purified γM proteins in complete Freund adjuvant until the antisera had high titers of antibody against γM globulin

Absorption — The anti γM antisera were absorbed with γG globulin in concentrations of 2—10 mg γG per ml of antiserum Whenever necessary these absorbed antisera were also absorbed with sera from agammaglobulinemic patients

In addition some of these antisera were subsequently absorbed with a monoclonal γM protein of the type different from that used for immunization

Tests — All antisera were tested by the Ouchterlony gel diffusion technique Purified γM proteins as well as whole sera were employed as antigens

Micro immunoelectrophoresis was performed as described elsewhere (2) either with agar or agarose gels

Results

Relatively few of the antisera produced could distinguish between the two types of γM proteins after absorption with γG Figure 1 illustrates the reaction of one such antiserum with normal serum γM and four sera from patients with macroglobulinemia of Waldenstrom The antiserum was against a type L γM protein and produced spurs whenever normal γM a mixture of γMK and γML was placed adjacent to a γMK containing serum This spur formation is of course due to anti L antibody that cannot combine with K molecules

Similar results were obtained with anti γMK sera normal γM and patients sera of type γML To date we have checked a large number of γM proteins of known type with these antisera and in each instance they reacted according to expectation — i e., proteins that reacted with antisera against kappa chains were always typed as K when checked with antisera against γMK and γML (absorbed with γG) and as yet we have not encountered any

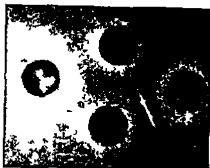


Figure 2 Ouchterlony gel diffusion plate Top serum from patient with monoclonal macroglobulinemia of type L (1 10) Bottom serum from patient with monoclonal macroglobulinemia of type K (1 10) Right specific anti γMK left specific anti γML

discrepancies between the two typing methods

Absorption of these anti γMK or anti γML antisera with γG and a monoclonal γM of opposite type resulted in specific antisera i e an anti γMK antiserum absorbed with a γML protein would react only with γMK molecules This is illustrated in figures 2 and 3 Note that the normal serum in figure 3 reacts with antisera against both types since it contains both γMK and γML Moreover the lack of any reactivity with γG or γA is apparent

To date all sera with antigenic monoclonal γM were obtained from patients with malignancies usually lymphomas We have encountered one serum from a patient with rheumatoid arthritis containing such large amounts of γM that it was indistinguishable from sera of patients with macroglobulinemia of Waldenstrom by ultracentrifugal analysis and paper electrophoresis Antigenically however, it was polyclonal i e both K and L γM types were elevated

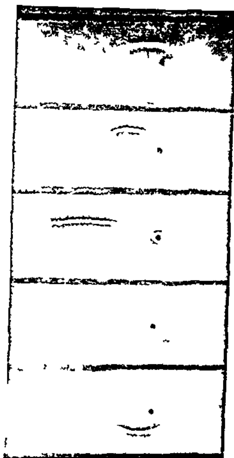


Figure 3 Immunoelectrophoresis on agarose gel Anode is on left The first three and the last hole contain sera from patients with monoclonal macroglobulinemia The fourth hole contains normal serum its γM globulin reacts with *both* specific anti γM sera The trenches contain alternately specific anti γML and anti γMK antisera Consequently the first serum contains a γML globulin Note that occasionally the patient s sera contain besides the monoclonal γM also some normal γM globulin of the other type

Discussion

Two forms of hyper γ globulinemia — the electrophoretically diffuse and the electrophoretically homogeneous (spike) form have been known for a long time These are now usually designated as polyclonal and monoclonal In the former the elevation of

immunoglobulins is presumed to be caused by the proliferation of most of the immunoglobulin forming cells in the latter, the increase in immunoglobulins is the product of the offspring of one such cell which has become the predominant cellular population The polyclonal gammapathies are usually a response to infectious or inflammatory diseases the monoclonal gammapathies to malignant transformation Consequently from a diagnostic point of view it is important to establish whether a hyper γ globulinemia is of the polyclonal or monoclonal variety In the γG gammapathies this may be accomplished by electrophoretic or immunoelectrophoretic methods The γM gammapathies are not as easily recognized since many monoclonal γM gammapathies contain only slight increases of γM globulin with frequent large increases of polyclonal γG globulins Consequently an immunological method capable of distinguishing between the different γM gammapathies will be of great diagnostic value More over studies on the composition of the immunoglobulins in the hyper γ globulinemias may shed light as to when (and why) a polyclonal gammapathy changes into a monoclonal gammapathy The preliminary data presented here show that it is possible to distinguish between the two antigenic types K and L of the γM globulins without any interference of the γG and γA globulins in the serum Our data are also of theoretical interest since the antisera used in this work cannot react with the light chains which usually determine the K or L specificity of an immunoglobulin This finding suggests that either the heavy (μ) chain of the γM protein contain a configuration characteristic of the K or L type or that the junction of the light chain to the μ chain results in a configuration that is determined both by the type of the light chain and the class of the heavy chain Preliminary data favor the latter explanation i e the junction of a kappa chain to a μ chain forms a configuration detectable by antibody against a γMK globulin that can no longer react either with the isolated heavy chain or light chains

Summary

Antisera against γM globulins of either type K or L were absorbed with γG globulins until they no longer reacted with γG or the light chains of the immunoglobulins Some of these antisera still could distinguish γM globulins of type K from those of type L These antisera are now being used to type sera from patients with suspected γM gammapathies to determine whether they are antigenically monoclonal or polyclonal *Preliminary data suggest* that those antibodies capable of distinguishing between the two types of γM globulin react with a specific site produced by the junction of the light chains with the heavy (μ) chain of the macroglobulin

Acknowledgements

The author wishes to acknowledge the technical assistance of Mr Raul Vidal Miss Heidemarie E Kerker and Mrs Karen R Cherns

References

1 Cohen S & Porter, R R Structure and biological activity of immunoglobulins *In* Advances in Immunology IV, Edited by F J Dixon & J H Humphrey New York/ London Academic Press 1964 p 287

2 Korngold L von Leeuwen G & Engle Jr R L Ann N Y Acad Sci 101 203 1962

3 Waldenstrom J Acta Med Scand., 117 216 1944

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From the Department of Clinical Chemistry Karolinska Institutet Serafimerlasarettet Stockholm Sweden (Head Rolf Blomstrand M D)

A Thin-Layer Gel Filtration Technique for Proteins

A Simple Clinical Method for Measuring Serum Macroglobulins

By KURT BERGSTRÖM[1]

Gel filtration has become an established technique for separating proteins according to molecular size (1 5 9) For analytical purposes thin layer chromatography techniques have been successfully applied especially since a loosely cross linked gel (Sephadex G 200 Superfine) with appropriate particle size has become available (6 7 8)

The most accurate way of confirming the diagnosis of macroglobulinemia is to measure the amount of macroglobulins by ultracentrifugation However gel filtration provides a less expensive and easier way of studying the protein molecules As with ultracentrifugation serum can be separated into three main fractions according to molecular weight (or volume) 4 5 S or the albumin group 7 S or the gamma globulin group and 19 S or the macroglobulin group

This paper is concerned with a thin layer gel filtration technique for quantitative study of these three groups of serum proteins It is recommended for quantitative studies of pathological sera as a simple and inexpensive complement to paper and immuno electrophoresis and also for estimating the molecular size of purified proteins

Materials and equipment

Gel Sephadex G 200 Superfine (lots To 33,4 and To 5761 both with particle size 10—40 µ) was obtained from Pharmacia AB Uppsala Sweden

Glass Plates Grooved commercial window glass available at any glaziers was cut the long way into 10×23 cm rectangles (cross section shown in Figure 1) Each plate had seven grooves 23 cm long which served the same purpose as stripping the supporting media in other chromatographic techniques

Reference Substances *Blue Dextran 2000* — average molecular weight 2 000 000 (Pharmacia AB) is used in solution (4 0 Gm/100 ml distilled water) *Trypsin* — Trypure® Novo Copenhagen Denmark. *Haemoglobin* — prepared by haemolyzing equine washed red blood cells and freeze-drying the clear supernatant after removal of the cell mem

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[1] Presently Visiting Assistant Professor Department of Medicine Blood Enzyme and Hematology Laboratory New York University Medical Center New York N Y U.S.A

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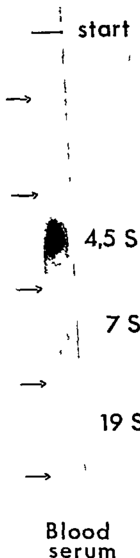


Figure 3 Stained filter paper strip with indications for cutting

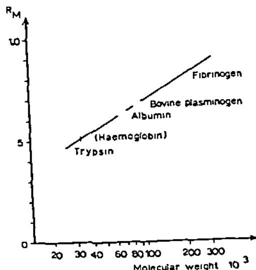


Figure 4 Curve obtained by plotting molecular weights of purified proteins against their R_M values Haemoglobin has an R_M value corresponding to a molecular weight of about 33 000

and directly stained for about 20 minutes in the Amido Black solution The excess stain is removed by repeated washings in the acetic acid water methanol solution For the last wash the strips are preferably left overnight in the solution After they are dried at room temperature the colored fractions are cut out and eluted The three protein fractions and a blank value are obtained by cutting pieces of *the same size from the paper (Figure 3) They* are eluted in glass stoppered test tubes by intermittent mixing for at least one hour When normal serum is analyzed 4 0 ml of the eluting so ution is used for the 4 5 S fraction and 1 0 ml for the others Absorbance of the protein fractions is read against the blank in semimicrocuvettes with a 10 mm pathway at 620 nm and the percentage of each fraction is determined from the amount of stain In calculating the approximate molecular size of a protein fraction, the migration distance from the starting point to the center of the spot (d_P) is divided by the distance traveled simultaneously by the macroglobulins (d_M)

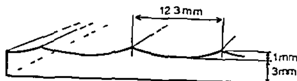


Figure 1 Cross section of glass plate for gel thin layer maximum depth of grooves is 1 0 mm

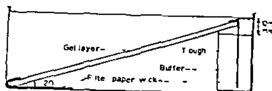


Figure 2 Vertical section of developing chamber Gel is layered on plate which is mounted as shown at an angle of about 20° Lower filter paper wick ensures more even flow

branes by centrifugation *Albumin* — bovine crystalline serum albumin Armour Pharmaceutical Co Kankakee Illinois USA *Bovine Plasminogen* — prepared by the author s method (2) *Fibrinogen* — bovine fraction I 4 99 % clottable prepared by the Blombäcks method (4)

Reagents Buffer consisting of 0 05 M tris and 1 M sodium chloride (pH adjusted to 8 0 with hydrochloric acid) was used throughout the procedure *Staining Solution for Paper Strips* — 0 5 Gm Amido Black 10 B in 10 ml glacial acetic acid 40 ml distilled water and 50 ml methanol *Wash Solution* — 10 ml glacial acetic acid 40 ml distilled water and 50 ml methanol *Eluting Solution* — 0 5 Gm Na_2EDTA 1 M NaOH (500 ml) and 95 % ethanol (500 ml)

Filter Paper Whatman No 3 MM

Transparent Plastic Box A bread box 7 5× 19 5×26 cm was used as a developing chamber with a glass trough about 4× 5×11 cm to hold the buffer (Figure 2)

Method

For most materials all operations can be carried out at room temperature In a filtering flask 4 0 Gm of Sephadex is mixed with 100 ml of buffer After the gel has been allowed to swell for 72 hours the entrapped air bubbles are removed by applying a vacuum (water suction pump) for about 15 minutes The Sephadex gel is poured onto the clean dry glass plate placed on a table and distributed evenly in the grooves with a thin smooth edged glass spreader about 5×15 cm The final layering is done by gently pulling the spreader lengthwise over the entire plate from one end of the glass to a point beyond the other end After 3 to 5 minutes the gel plate is suspended in a transparent plastic box (a level is used to ensure a perfectly flat surface) and equilibrated for at least 12 hours The plate arrangement is shown in Figure 2 A wick of triple folded filter paper which sucks the buffer up onto the plate should extend about 10 mm onto the gel layer At the start of the chromatography the top of the wick should be 10—15 mm above the buffer level in the trough to provide a suitable flow rate The glass trough contains 150—200 ml of buffer Slightly less than 2 0 μl of test solution (20 mg/ml) or undiluted serum is carefully deposited in the middle of each groove with a 2 0 μl semiconstriction micro pipette 20 mm from the plate s upper edge It is important to avoid scratching the gel layer or blowing air through the pipette Blue Dextran 2000 applied to one of the grooves acts as an indicator of the flow rate The blue spot can easily be seen through the transparent box lid against a white background

Development requires about 5 hours The chromatography is considered complete when the Blue Dextran spot is observed about 40 cm from the lower end of the plate The filter paper wick is then carefully removed and the gel plate is placed on a flat glass surface for 3 to 4 minutes The grooves are covered with previously prepared strips of filter paper 10×300 mm which are rounded horizontally with the fingers to facilitate progressive adhesion from the centerline of each groove to the remaining gel surface After the filter paper strips are allowed to absorb the solution from the gel for 5 minutes they are removed

Discussion

The thin layer gel filtration technique described here is used to separate proteins according to molecular size (volume). The contribution of lipoproteins to the normal macroglobulin value has been disregarded. For this reason and because of certain technical details the results cannot be directly compared with ultracentrifuge analyses. This quantitative method however provides a simple means of estimating the amount of macroglobulins in a patient's serum. The mean value found for macroglobulins was 5.7 % and the coefficient of variance was 0.31 — relatively high compared to 0.04 for the albumin group. In none of the normal sera however did the macroglobulin value exceed 10 % of the total *serum protein* (duplicate analyses).

The technique is clinically useful to determine whether an M component detected by paper electrophoresis consists of macroglobulins or 7 S gamma globulins. The method has also been used for comparative study of serum proteins from human lymph and blood (3).

Summary

A thin layer gel filtration technique for proteins is described. It requires no special layering equipment and permits determination of the molecular size of purified protein fractions. This quantitative method has been used to determine normal values for human serum protein fractions. It is suggested as a valuable complement to paper and immuno electrophoresis in clinical applications.

References

1. Andrews P. Biochem J *91* 222 1964
2. Bergstrom K. Arkiv Kemi *21* 517 1963
3. *Bergstrom, K. & Werner B.* Acta Chir Scand In print 1965
4. Blomback B. & Blomback M. Arkiv Kemi *10* 415 1956
5. Flodin P. & Killander J. Biochim Biophys Acta 63 403 1962
6. Grant G. H. & Everall P. H. J Clin Path *18* 654 1965
7. Johansson B. G. & Rymo L. Acta Chem Scand *18* 217 1964
8. Morris C. J. O. R. J Chromatog *16* 167 1964
9. Whitaker J. R. Anal Chem 35 1950 1963

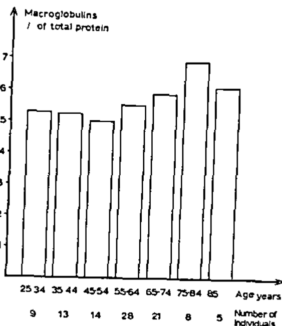


Figure 5 Macroglobulin values for different age groups

in normal human serum. A standard curve is prepared by plotting the ratio (Rg=dp/dg) for proteins with a known molecular weight (Figure 4).

Table 1 A single serum sample analyzed twelve times: mean, standard deviation, and coefficient of variance are calculated for each protein group

Group of proteins	4.5 S	7 S	19 S
Mean %	69.6	25.0	5.4
Standard deviation	2.8	2.7	1.7
Coefficient of variance	0.04	0.11	0.31

Table 2 Mean values, standard deviations and approximate normal limits for practical use (about M±2s) are calculated from 204 separate determinations (two analyses on each of 102 different normal serum samples)

Group of proteins	4.5 S	7 S	19 S
Mean (M) %	69.7	24.6	5.7
Standard deviation (s)	5.4	4.8	2.0
Approximate normal limits	60—80	15—35	<10

Results

Results were not affected by the use of serum samples kept at —20° C up to one month. Reproducibility of the method was tested by analyzing one sample of normal human serum 12 times (Table 1). Normal values for the three groups of proteins in human serum were obtained by running two analyses on serum from each of 102 subjects — a total of 204 on which the calculations were based. Total protein values and results of paper electrophoresis in these persons were in the normal range. The mean values, standard deviations and approximate normal limits (M±2s) for the different fractions are shown in Table 2. The 19 S group of proteins did not account for more than 10 % of the total protein in any of the normal sera. When the macroglobulin values were grouped according to the subjects' ages the means varied between 5 and 7 % with the higher values in the older age group (Figure 5).

Some purified proteins were found to give a straight line when the Rg values were plotted against the log for their respective molecular weights (Figure 4). Haemoglobin behaves differently showing a molecular weight of only about 33 000 — a finding reported by several other investigators using similar methods (8).

cultures of peripheral blood or bone marrow from patients with Macroglobulinemia Waldenstrom (2 3 9 10 11, 14 17 21) The occurrence of this large supernumerary chromosome seems to be limited to the cells of the lymphoplasmoreticular system — in other words — to the tissue which has undergone neoplastic changes *Ferguson and Mackay* (10) were unable to demonstrate this abnormal chromosome in fibroblast cultures of their patient Whereas this extrachromosome of group A was at first described only in patients with Morbus Waldenstrom it has now also been demonstrated in patients with multiple myeloma (24 25 26) It represents the most frequent and outstanding chromosomal finding in paraproteinemic diseases Today a distinct probability exists of a pathogenetic relationship between this striking extrachromosome and the development of these neoplasms *Waldenström* (30) has indicated that with the acceptance of *Burnet's* clonal conception one must postulate that the responsible genetic make up of the individual is either present from birth through inheritance or is acquired through mutation in later life The question is important whether the extrachromosome of group A seen in patients with Morbus Waldenstrom or myeloma represents a *specific abnormality or is the* consequence of a mutant gene which leads to mitotic errors but has no connection with the neoplastic disease

In the course of family examinations among blood relatives of patients with Macroglobulinemia Waldenstrom or myeloma, we have found one case which may help clarify the above problem

Case report

Our patient Margrit K born in 1906 was diagnosed as having Macroglobulinemia Waldenstrom in 1952 The 13 year course has been extremely benign and the general condition of this woman has remained relatively stable throughout this long time of observation The pathological findings are limited to the following a moderate anemia of about 11 g% Hb an elevated sedimentation rate of 100/110 mm a pronounced γM paraproteinemia (see fig 1 and 2) and cryoglobulinemia to which a large 16 1 S component seen in the ultracentrifugation corresponds characteristic changes of the bone marrow with infiltration with lymphocytes and large lymphoid reticulum cells as well as a light increase in tissue mast cells Despite a marked decrease of the normal immune globulins only mild clinical symptoms due to antibody deficiency have become manifest The detailed findings of this case have been reported elsewhere (26)

Both parents are deceased there was no evidence of consanguinity All of the eight siblings of our patient are still alive including her twin sister In none of the five sisters and three brothers were we able by means of thorough electrophoretic and immunochemical examinations to substantiate any criteria for the existence of Morbus Waldenstrom myeloma or asymptomatic ("idiopathic") paraproteinemia This was also true in the case of our patient's twin sister

Genetic examinations

We have gone to great lengths to establish the possible monozygotic relationship of our patient with paraproteinemic disease (Morbus Waldenstrom) and her healthy non paraproteinemic twin Information concerning the foetal membranes is not available due to the fact that the birth had taken place at

Institut fur klinische Eiweißforschung der Universität Bern (Direktor Prof G Riva) und Medizinische Poliklinik der Universität Tubingen (Direktor Prof F Heni)

Chromosomal abnormalities in Macroglobulinemia Waldenstrom Discordant findings in uniovular twins

By G A SPENGLER,[1] H SIEBNER[2] and G RIVA

An increased familial incidence of Macroglobulinemia Waldenstrom or multiple myeloma has been repeatedly reported Also an increase of other neoplastic diseases has been noted among the members of such families (27) *Seligmann* and co workers (23) are of the opinion that the incidence of paraproteinemia amongst blood relatives is higher than one would statistically expect in these relatively rare diseases (31) Therefore it would not be incongruous to postulate a genetic factor which may produce a hereditary predisposition to neoplastic changes such as those seen in the lymphoplasmoreticular system in cases of Morbus Waldenstrom, myeloma etc

The pathogenesis of the paraproteinemic diseases is perhaps best explained by *Burnet* (4) who postulates a *mutation* as the basis of these neoplasms Originally this mutation affects only a single cell but persists in the descendants of this cell "*Burnet* who thinks in terms of a bacteriologist compares this process with the growth of a bacterial colony in other words all plasmocytoma cells are descendants of a specifically different ancestor cell" (30) The same pathogenetic mechanism may also be considered valid for Morbus Waldenström The theory of the monoclonal genesis of paraproteinemic diseases was accepted and enlarged upon by *Waldenström* With his concept of *monoclonal hypergammaglobulinemia* (29 30) he furthered the accepted principle that paraproteins are qualitative and/or quantitative abnormal variants of the immunoglobulins (13 22) Through recent findings *Burnet s* postulate which bases the origin of paraproteinemic neoplasms on the mutation of a single cell of the lymphoplasmoreticular system has acquired a new aspect

Numerous authors have demonstrated the presence of an *extrachromosome of group* I in mitoses seen in

[1] Supported by a grant of the Schweizerischer Nationalfonds zur Forderung der wissenschaftlichen Forschung

[2] Supported by a grant of the Deutsche Forschungsgemeinschaft

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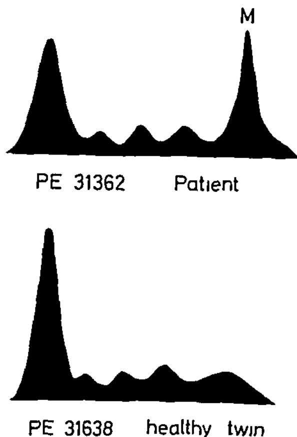


Fig 1 Electrophoretic serum protein patterns of the monozygotic twins

Upper diagram Our patient (Margrit K) with macroglobulinemia Waldenstrom shows a distinct M gradient (37 rel %) in γ position corresponding to the γM paraproteinemia Total serum protein 9 75 g% viscosity 6 4 ESR 100/110 mm

Lower diagram Her healthy uniovular twin sister (Johanna K) shows an inconspicuous pattern Total serum protein 6 75 g% viscosity 1 7 ESR 9/17 mm

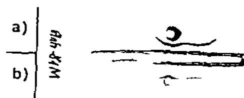


Fig 2 Immunoelectrophoretic findings in the monozygotic twins

a) Serum (undiluted) of our patient with γM paraproteinemia (Morbus Waldenstrom) anti γM immunoglobulin antiserum Only the accentuated γM precipitation line demonstrating a double inflection and pronounced bends at both ends and parts of the macroglobulin fraction which have been precipitated round the serum deposit site have developed

b) Serum (undiluted) of the healthy twin sister anti γM immunoglobulin antiserum Only the normal γM precipitation line has developed

home and the attending midwife has since died Therefore we were dependent upon indirect methods

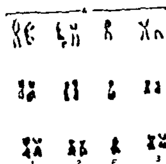
The *phenotype* of the twin sisters has been remarkably similar since childhood and is even more outstanding when compared to that of the other siblings General body frame hair and eye colours as well as clothing and shoe sizes are identical Inasmuch as both sisters are single and have no children corresponding examinations of descendants are out of question

Blood and serum groups (ABO Rh CDE cw MNS P_1 Kell Duffy haptoglobin Gc Gm Inv) were determined in all siblings Whereas in the case of the twins all these factors are identical many variations exist among the other brothers and sisters

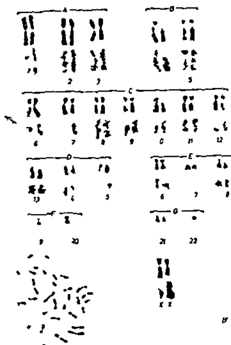
Contrary to the rather common belief existing among both laymen and physicians *hand and fingerprint patterns* of uniovular siblings are by no means identical but may show great variations Nevertheless fine details of the print patterns may be used to substantiate the fact of monozygotism Appropriate criteria as defined by *Maynard Smith* and co workers (16) were studied in our twin sisters

The *statistical evaluation* of all our findings (blood and serum groups of all the siblings hand and fingerprint patterns of the twin sisters) was carried out by Professor S *Rosin*[1] and his co worker Miss *F Schmid* in a manner we greatly appreciated It was

[1] Chief of the Division of Hereditary Research at the Zoological Institute of the University of Bern



F g 3 Autosomal groups A of three m toses w th 47 chron osomes from our pat ent (Marg r t h) with Morbus Waldenstrom The extra chromoson e E f ts between the autosomal pa rs No 2 and 3



F g 4 Normal karyotype of tl e healthy tw n s ster (Johanna K)

Upper rows Cl ron osomes sta ned w th hematoxyl n

Lower ro vs Autorad ograpl c pattern (late pl ase) of the same cl ro nosou es n arked w th tr t ated thyn d n Chromosomes sta ned w th G e nsa

shown that tl e probab l ty that the s sters were ndeed monozygous ex sted w th a certa nty between 99.8190 % (probab l ty of error of tl s statement 0 1810 %) and 99 9 9 % probab l ty of error 0 0008 % The most probable alue s 99 9 88 % (probab l ty of error 0 0?1? %) W th th s we l ave the tat t c assurance that our pat ent and her ster are u o ular tw n s The pa ns taken to ol ta n th s proof was necessary for the ub equent nterpretat on of our *chromosomal stud es* un lertaken n these two won en

In our pat ent Marg r t h with Morbus Waldenstrom a cl aracter st c large *extr h omosome of group A* (6 was found in a port on of the n etaphases of her blood and bone marrow cultures

Accord ng to ts s ze th s add t onal chro mo o ne f ts between tl e autosomal pa rs No an l 3 and possesses a med an to submed an entr n ere Tl e ex stence of an abnormal X cl o o ne was ruled out through the f nd n f no al sex chromat n patterns n the buccal sn ear and tl e pre ence of drum ks n tl e leukoc tes Also the e aluat on f tl e autorad ograpl c f ln s showed no e lence of a supernun erary late labell ng X chron oso e In add t on n some m toses the

cl ron o omes of group A demonstrated a stretch g of the centromere reg on wl ch was not ced n pat ents w th Morbus Walden *strom* as well as n norn al nd duals (1o 24—26 Furtl ermore our pat ent — n addi t on to her cell l ne w th the supernun erary chromoson e of group A — also l o *ed an* other cell l ne w th 47 cl romosomes Th s l ne was characterized by a small almost meta centr c extrachron osome of a s ze *correspond in* to tl e autosomes No 20 (group F) As a rule one cons ders tl e presence of super *numerary small autosomes* espec ally those of group G as a s gn of ncreased mal gnancy n cases of neoplastic paraprote nem a (6 26)

Table 1 Number of chromosomes in peripheral blood (72 hours of incubation) and bone marrow (7 hours of incubation) from the uniovular sisters (*italic numbers* = mitoses which were analyzed in detail with the aid of photographic mountings)

Number of chromosomes	< 43	43	44	45	46	47	> 47	Total of counted mitoses
patient (Margrit K.)								
blood culture	2	1	2	4	57	9	1	76
					19	*9*		*28*
marrow culture			1	2	15	4		22
					3	*4*		*7*
healthy twin sister								
1st blood culture				4	46			50
					17			*17*
2nd blood culture					10			10
					6			*6*

Despite this, our patient has demonstrated a particularly benign course of Morbus Waldenström. In this case one could assume that the "trisomy F" originated from a cell with the large extrachromosome of group A which has lost the greater part of its long and short arms through deletion.

When cultures of peripheral blood from our patient were treated with tritiated thymidin, the metaphase figures with 47 chromosomes showed either a very late stage of replicating DNA (with a "hot" X chromosome and with only few silvergrains over the autosomal set including the extrachromosome) or were not labelled at all. Thus in this patient we do not know the DNA replicating pattern of the extrachromosome up to now.

In the case of our patient's monozygotic twin sister who is clinically healthy and shows no abnormalities in her serum protein pattern, repeated studies failed to reveal the presence of an extrachromosome of group A. Instead, the evaluation of a total of sixty metaphases derived from two cultures of peripheral blood consistently showed a numerically normal chromosome complement. However, this healthy twin did demonstrate an abnormal frequency of chromatid breakage. This mostly involved the autosome pair No. 3 and could be noted in 24 to 30 % of the cells.

According to the findings of the Edinburgh group (7, 8, 28), stable and unstable aberrations may be found in 1.4 % of the cells of blood cultures taken from normal adults. This finding corresponds well with our experiences. Today, on the basis of clinical and experimental observations, viral infections of the patients or the culture media have been primarily incriminated as the cause of highly increased rates of chromatid breakage seen in blood cultures (1, 18, 19, 20). At the time the first blood culture was drawn from the normal twin sister, she was indeed suffering from an acute flu syndrome. It was for this reason that we repeated the examination four months later. Despite the fact that this second culture again showed increased breaks, we would prefer not to interpret this as an inborn anomaly, for such an increase in breaks was not present in the twin sister with Morbus Waldenström despite her tendency toward chromosomal abnormalities as demonstrated by her cell line with 47 chromosomes. We also do not believe that this increase of chromatid breaks results merely from adding tritium labelled thymidin, inasmuch as they were also seen in unlabelled mitoses as well as in the control culture without tritiated thymidin.

Autoradiographs of ^{3}H thymidin marked

chromosomes showed a normal DNA replication pattern for the healthy twin sister with her numerically normal chromosomes (see fig 4)

Discussion

We have now followed a patient with typical Morbus Waldenström for 13 years She demonstrates γM paraproteinemia and characteristic bone marrow findings, whereas the clinical course to date has been extremely benign In our opinion this case is unique and of particular interest inasmuch as she has a twin sister whom we have been able to examine thoroughly Contrary to our patient this twin sister demonstrates none of the criteria for paraproteinemic disease and is clinically healthy Statistical evaluation of data obtained from blood and serum group studies as well as palm and finger print examinations have shown these sisters to be of monozygotic origin with a certainty of 99 9788 % It follows that comparative chromosomal studies in this uniovular set of twins could be of paramount interest

The fact that we could demonstrate a large extrachromosome of group A which was not of X chromosomal origin in the cultures from peripheral blood and bone marrow of our patient with Morbus Waldenstrom agrees well with the majority of the current literature reports as well as with our previous findings In contrast to this careful evaluation of 60 metaphases from two separate blood cultures obtained from the healthy monozygotic twin failed to demonstrate the presence of such an extrachromosome — the chromosome complement of this woman was numerically normal

Inasmuch as monozygotic twins primarily possess identical genetic make up and therefore identical chromosomal patterns too one must assume that the extrachromosome of group A which was found only in the twin sister with Morbus Waldenstrom represents an acquired chromosomal mutation a fact which is proved by the presence of a mosaic of normal/abnormal cells Therefore it seems that a direct pathogenetic correlation exists between this acquired chromosomal mutation and the paraproteinemic neoplasm (Morbus Waldenström myeloma) The fact that many patients with macroglobulinemia or myeloma have demonstrated a similar chromosomal abnormality namely an extra chromosome of group A is in our opinion worth emphasis For each individual patient this supernumerary chromosome represents a new acquisition which must be explained through a noxa effecting a strikingly similar aberration of the chromosomal complement This aberration is apparently seen only in the tissue that has undergone neoplastic degeneration in other words in cells of the lymphoplasmoreticular system As yet it is impossible to say whether either the paraprotein synthesis or the cellular neoplastic proliferation or both these phenomena together are dependent upon the presence of the additional chromosome of group A If one supposes that in the increased familial incidence of paraproteinemic diseases

hereditary factors do indeed play a role, other modes of genetic transmission must be postulated, such as possibly through a recessive autosomal gene (23), perhaps predisposing to mitotic errors of lymphoplasmoreticular cells

In conclusion it should be noted that the findings herewith reported lie in the realm of rare natural experimentation The findings also contain the pitfalls of all single observations We would therefore like to call attention to the report of *Goh* and *Swisher* (12) which describes a male patient with chronic myelocytic leukemia in whom the typical Philadelphia chromosome was demonstrated Here also a clinically healthy, monozygotic twin failed to demonstrate a chromosomal abnormality This case which is most comparable to that of our twin sisters seems to substantiate our findings and conclusions

Summary

A case is described concerning a female patient who has suffered for 13 years from Morbus Waldenstrom (benign form) with mild anemia extremely increased ESR γM paraproteinemia (macroglobulinemia), and typical bone marrow changes In cultures from her peripheral blood and bone marrow a large extrachromosome of group A could be demonstrated in a portion of the metaphases such as seen previously in other patients with paraproteinemic neoplasms Our patient s twin — the monozygotic origin of the sisters could be statistically proved with a certainty of 99 9788 % — is clinically healthy and shows no symptoms of a paraproteinemic disease In two different blood cultures from the normal twin sister no extrachromosome could be demonstrated, and the chromosomal pattern was numerically normal The extrachromosome of group A in our patient with Morbus Waldenström must therefore be regarded as an acquired chromosomal mutation A direct *pathogenetic correlation between the* occurrence of this chromosomal aberration and the paraproteinemic disease seems to be proved

Acknowledgements

We should like to express our appreciation to Dr R Bütler Chief of the Serological Department Central Laboratory of the Blood Transfusion Service of the Swiss Red Cross in Bern (Director PD Dr A Hassig) to whom we owe numerous immunochemical examinations as well as the determination of blood and serum groups We are indepled to Dr Regula Gloor (Genetic Laboratory Bernese Women s Hospital) for the evaluation of hand and finger prints

References

1 Aula P Lancet 1964/I 720

2 Benirschke K Brownhill L and Ebaugh F G Lancet 1962 I 594

3 Bottura C Ferrari J und Veiga A A Lancet 1961 I 1170

4 Burnet M The clonal selection theory of antibody production Oxford Univ Press London and New York 1959

5 Braunsteiner H Rothenbuchner G and Schober B Wien klin Wschr *76* 502 (1964)

6 Castoldi G L Haematologica *40* 751 (1964)

7 Court Brown W M Buckton K E and McLean A S Lancet 1965 I 1399

8 Court Brown W M Buckton, K E., and McLean A S Lancet 1965/I 1239
9 Elves M W and Israels M C G Brit med J 1963/II 1024
10 Ferguson J and Mackay I R Aust. Ann Med *12* 197 (1963)
11 German J L Biro C. E and Bearn A G Lancet 1961/*II* 48
12 Goh, K O and Swisher S N Arch Intern Med *115* 475 (1965)
13 Hassig A Ergebn Mikrobiol *34* 180 (1961)
14 *Heni F and Siebner H Dtsch med* Wschr *88* 1781 (1963)
15 Herreros B Ortiz O Banuelos J Velasco R and Bosque P G Lancet 1964/I 557
16 Maynard Smith S Penrose L S and Smith C A B Mathematical tables for research workers in human genetics Churchill London 1961
17 Moretti G Hartmann L., Staeffen J., de Grouchy J Catanzano G and Broustet A *Ann Génet 8 55 (1965)*
18 Nichols W W Levan A Coriell, L. L., Goldner H and Ahlstrom C G Science *146* 248 (1964)
19 Patau K Lancet 1961/II 600
20 Patau K Pathologie Biologie *11* 1163 (1963)
21 Pfeiffer R A., Kosenow W., and Bäumer A Klin Wschr *40* 342 (1962)
22 Roulet, D L. A., Spengler G A., Gugler E Bütler R Ricci C., Riva G., and Hassig A Helv med Acta *28* 1 (1961)
23 Seligmann M Fine J M and Danon F Protides of the Biological Fluids XIth *Colloquium 1963 (Bruges)* p *78—85* Elsevier Amsterdam London New York 1964
24 Siebner H and Spengler G A Proceedings of the Symposium on Mutational Process (Prag 16—18 8 1965) (in press)
25 Siebner H and Spengler G A Kongressbericht der 9 Tagung der Deutschen Gesellschaft fur Anthropologie (Freiburg i Br., 11 10 1965) (in press)
26 Siebner H., Spengler G A., Butler R Heni, F., and Riva G Schweiz med. Wschr 95 1767 (1965)
27 Spengler G A Butler R., Fischer Ch Ryssel, H J and Siebner H Helv med Acta *32* 449 (1965) ibid *33* (1966) (in press)
28 Tough, J M and Court Brown W M *Lancet 1965/I* 684
29 Waldenstrom J Harvey Lecture 56 p 211—231 Academic Press Inc New York 1961
30 Waldenstrom J Progr Allergy *6* 320 (1962)
31 Waldenstrom J Med Klin *59* 413 (1964)

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In conclusion it should be noted that the findings herewith reported lie in the realm of rare natural experimentation The findings also contain the pitfalls of all single observations We would therefore like to call attention to the report of *Goh* and *Swisher* (12) which describes a male patient with chronic myelocytic leukemia in whom the typical Philadelphia chromosome was demonstrated Here also a clinically healthy, monozygotic twin failed to demonstrate a chromosomal abnormality This case which is most comparable to that of our twin sisters seems to substantiate our findings and conclusions

Summary

A case is described concerning a female patient who has suffered for 13 years from Morbus Waldenstrom (benign form) with mild anemia, extremely increased ESR γM paraproteinemia (macroglobulinemia) and typical bone marrow changes In cultures from her peripheral blood and bone marrow a large extrachromosome of group A could be demonstrated in a portion of the metaphases such as seen previously in other patients with paraproteinemic neoplasms Our patient s twin — the monozygotic origin of the sisters could be statistically proved with a certainty of 99 9788 % — is clinically healthy and shows no symptoms of a paraproteinemic disease In two different blood cultures from the normal twin sister no extrachromosome could be demonstrated, and the chromosomal pattern was numerically normal The extrachromosome of group A in our patient with Morbus Waldenström must therefore be regarded as an acquired chromosomal mutation A direct pathogenetic correlation between the occurrence of this chromosomal aberration and the paraproteinemic disease seems to be proved

Acknowledgements

We should like to express our appreciation to Dr R Butler Chief of the Serological Department Central Laboratory of the Blood Transfusion Service of the Swiss Red Cross in Bern (Director PD Dr A Hassig) to whom we owe numerous immunochemical examinations as well as the determination of blood and serum groups We are indepted to Dr Regula Gloor (Genetic Laboratory Bernese Women s Hospital) for the evaluation of hand and finger prints

References

1 Aula P Lancet 1964/I 720
2 Benirschke K Brownhill I and Ebaugh F G Lancet 1962 I 594
3 Bottura C Ferrari J und Veiga A A Lancet 1961 I 1170
4 Burnet M The clonal selection theory of antibody production Oxford Univ Press London and New York 1959
5 Braunsteiner H Rothenbuchner G and Schober B Wien klin Wschr 76 502 (1964)
6 Castoldi G L Haematologica 40 751 (1964)
7 Court Brown W M Buckton K F and McLean A S Lancet 1965 I 1399

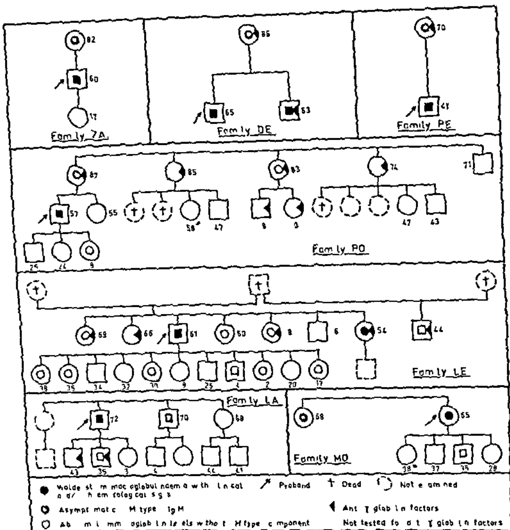


Fig 1

two and three years after the first serum examination the sharp Ig M peak was no longer found on agar gel electrophoresis the ultracentrifugal patterns showed only 3 % heavy components and Ig M of both light chain antigenic types were present (3) Chromosomal studies performed by J Lejeune and J Tanzer on most of these patients and relatives with macroglobulinaemia have shown no large extra chromosome similar to that described by Bottura and al (4) except in a few cells of patient LA Detailed clinical data on some of the probands can be found elsewhere (2) and (5) for ZA (1) for DE (6) for LE

A comparative study of the macro

From the Department of Immunochemistry of the Research Institute o
Blood diseases, University of Paris — Hopital Saint Louis

A Genetic Predisposition to Waldenstrom's Macroglobulinaemia

By Maxime Seligmann

The finding of a familial occurrence of macroglobulinaemia in two families (1, 2) has stimulated an investigation of relatives of patients with Waldenstrom's macroglobulinaemia (W.M.). This systematic family survey has been accomplished with Drs Françoise Danon, H. Fudenberg and C. Mihaesco, and this paper gives a short summary of our results.

We have studied the sera of 216 close relatives (192 first degree) of 65 patients. This investigation is somewhat limited since in 35 families we were able to study only one or two first degree relatives. All the probands had the signs of W.M. with a lymphoid infiltration of the bone marrow and more than 25 % heavy components on ultracentrifugation of the serum.

The three main abnormalities found in these families are: 1) presence of an IgM type "M component" with or without evidence of disease, 2) abnormal immunoglobulin levels without detectable "M component", 3) occurrence of anti-γ globulin factors in non elderly relatives.

The familial occurrence of IgM type "M-components"

As shown in Fig. 1, this has been found in 7 out of the 65 families. There was no evidence of consanguinity in these 7 families. Typical W.M. occurred in two siblings in each of two families. A significant increase of IgM associated with a sharp band on electrophoresis has been found in 6 apparently healthy relatives: 4 mothers and 2 siblings. Clinical examination and blood counts were normal in these asymptomatic relatives. A sternal puncture was performed in only one of them and showed no abnormality. The percentages of heavy components on ultracentrifugation of these 6 sera were respectively 6, 9, 9, 12, 12 and 18 %. The IgG and IgA levels were normal in four of these sera, significantly increased in one and greatly decreased in one. In the mother of family DE the IgM "monoclonal" component was transient

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[1] Supported in part by Grant n° 61 FR 062 of the Fonds de Developpement de la Recherche Scientifique

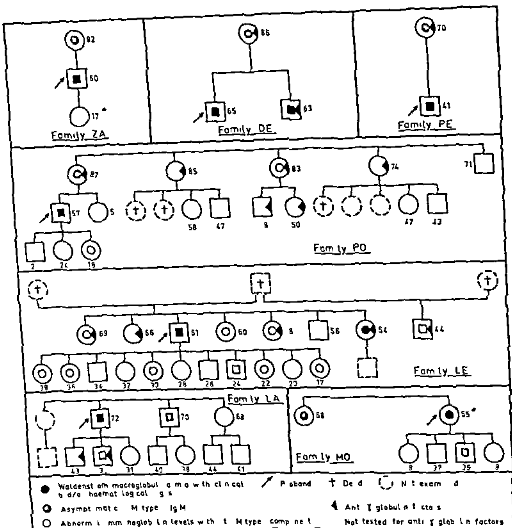


Fig 1

two and three years after the first serum examination the sharp Ig M peak was no longer found on agar gel electrophoresis the ultracentrifugal patterns showed only 3 % heavy components and I_{g} M of both light chain antigenic types were present (3) Chromosomal studies performed by J Lejeune and J Tanzer on most of these patients and relatives with mac roglobulmaemia have shown no large extra chromosome similar to that described by Bottura and al (4) except in a few cells of patient LA Detailed clinical data on some of the probands can be found elsewhere (2) and (ɔ) for ZA (1) for DL (6) for LE

A comparative study of the macro

Table I Abnormal immunoglobulin levels in first degree relatives of patients with Waldenstrom macroglobulinaemia

	No tested	High IgM "M type	High IgM			Low IgM			High IgA		Low IgA		Total abnormal	
			1	with high IgG	with high IgG and IgA	1	with low IgA	with high IgG and IgA	1	with high IgG	1	with low IgG	No	%
Parents	18	4	1	1	—	2	1	1	2	—	—	1	13	72%
Siblings	68	4	3	—	—	10	2	—	9	2	5	—	35	52%
Offsprings	106	—	6	1	3	6	4	—	6	—	10	1	37	35%
Total	192	8	10	2	3	18	7	1	17	2	15	2	85	44%

[1] Normal levels for the 2 other immunoglobulins

globulins in different affected members of the same family has been performed Some differences have been found within the same family in *electrophoretic mobilities sedimentation* constants, and Inv factors on the purified Ig M Starch gel electrophoresis of reduced and alkylated Ig M showed that the mobility of the light chains was different in the affected members of the 3 families studied The individual specific antigenic structure of the macroglobulins has been shown to be different in patient PE and his mother in patient LE and his sister, in patient LA and his brother and in the two brothers DE Studies with individual specific antisera to the Ig M of each of these DE brothers have shown that their mother s serum contained two antigenically distinct macroglobulins one with an antigenic structure similar to the macroglobulin of the first son and the other similar to that of the second son (7) No satisfactory explanation has been found for this peculiar finding

Abnormal immunoglobulin levels without detectable "M component"

Immunoglobulin levels have been roughly estimated by immunoelectrophoretic analysis with different quantities of serum, and quantitative determinations have been performed by a method using radial diffusion in antibody agar plates (8) The results obtained with random samples of a normal population have led us to consider as abnormal" the *following* concentrations for Ig G < 7 mg/ml or > 18 mg/ml for Ig A < 1 mg/ml or > 3 mg/ml for Ig M < 0 6 mg/ml or > 2 mg/ml Although we did not study *matched control families* the overall incidence of immunoglobulin concentrations differing from the normal values is high in the first degree relatives of patients with W M (Table I) Abnormal immunoglobulin levels have been found in 45 of the 65 families and in 24 of the 30 families which included more *than 2 first degree* relatives As seen in Table I abnormal Ig M and/or Ig A levels are more fre

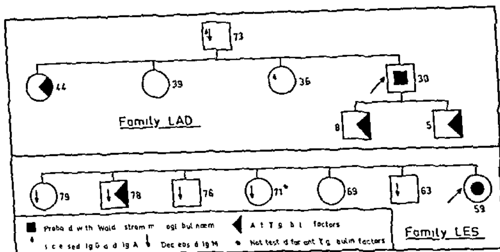


Fig 2

quent than abnormal Ig G levels Many different combinations of ab normalities are encountered (see for example in Fig 2 the father in family LAD) We have never found an important decrease of all 3 immuno globulins nor an absence of any one immunoglobulin Low Ig M levels are more frequent than diffuse hyper Ig M globulinaemia In family LES (Fig 2) 5 of 6 siblings have a marked decrease of Ig M The high incidence of abnormal levels in the probands parents must be emphasized In siblings and offsprings the occurrence of abnormal immunoglobulin levels is independent of the sex None of the offspring with abnormal levels was less than 18 years old

Anti γ globulin factors

The results of the tests with human and rabbit γ globulins are given in Table II Positive reactions have been found in 33 of 60 families and in 28 of 42 families with two or more studied relatives The results are not related to the sex of the proband nor of the relatives The incidence of positive reactions with either test in the elderly relatives is not significantly different from the findings in normal controls but the incidence is abnormally high *in the relatives* less than 60 years old The two "sero positive" boys in family LAD (Fig 2) respectively 5 and 8 years old had never received transfusions nor γ globulin injections There is no correlation between the Ig M level and the positive serological reactions anti γ globulin factors were found in only 7 of the 14 relatives with diffuse hyper Ig M globulinaemia the Ig M level was normal in 60 % of the sera with positive anti γ globulin reactions and low in 3 cases

Table II Incidence of anti γ globulin factors in first degree relatives of patient with Waldenstrom macroglobulinaemia

Age groups	0—20	20—40	40—60	60—70	> 70	Total relative
No tested	10	74	45	22	20	171
No positive reactions with						
Rabbit γ globulin	1	9	9	2	3	24
Human γ globulin [1]	1	9	14	3	6	33
No positive reactions with both tests	0	3	6	1	1	11
No of sera positive with either test	2 (20 %)	15 (20 %)	17 (38 %)	4 (18 %)	8 (40 %)	46 (2. %)

[1] = Ripley coat

Other antibodies

The results are essentially negative

Cold agglutinin titers have been measured in the sera of 157 relatives a titer of 1/16 was found in four sera and of 1/128 in two

Antinuclear factors have been investigated in 196 sera The incidence of positive reactions was slightly higher than in a control group matched for sex and age but the difference was not significant and the titers were in general very low Only 4 sera were positive at a titer of 1/50 and one serum had a titer of more than 1/100 This was from a 21 years old daughter of a W M patient She showed no clinical abnormalities but her serum had very high levels of the three immunoglobulins and gave strongly positive anti human γ globulin reactions serological tests for syphilis were negative

The incidence of anti thyroid and anti gastric auto antibodies was lower than found in a control group

Discussion

Although the studies have been limited to a small number of relatives in many families they have shown a high incidence of quantitative and qualitative immunoglobulin abnormalities Therefore the findings strongly suggest a genetic background in at least some cases of W M Whether this genetic predisposition applies to all cases of W M or only to a subgroup within what may prove not to be a single disease is a matter of speculation

An IgM type "M component" has been found in 8 of 216 relatives six of whom were apparently healthy This is a high incidence compared to the 1 % frequency of all types of M components in a Swedish normal population above 25 years old (9) All of these 8 relatives were more than 50 years old and 4 of the 14 still alive

probands mothers showed an asymptomatic "monoclonal" Ig M component The frequency of so called "essential M components" is known to increase with age (10) However in a control group of 100 healthy French women above 70 years old we have found only one Ig G type and no Ig M type "M components" and Hallen reports only one Ig M type "M component" in his series of 294 healthy people above 70 years old The globulin abnormalities seems to become apparent only late in life in genetically predisposed individuals suggesting the influence of ageing and possibly of triggering environmental factors We do not know if the relatives with asymptomatic macroglobulinaemia will later develop the progressive malignant disease Some of these apparently healthy relatives have a rather high percentage of macroglobulin but on the other hand the "M component" has spontaneously disappeared in one mother The sharp distinction between "benign essential monoclonal hyperglobulinaemia" and multiple myeloma (11) probably does not hold for macroglobulinaemia since as Waldenstrom we have observed transitional forms

The nature of the genetically determined disorder(s) cannot be deduced from the available data The hypothesis of a structural gene abnormality is ruled out by the demonstration of a different biochemical and antigenic structure of the macroglobulin in affected members of the same family and besides there is no proof that Ig M of W M are truly abnormal molecules Moreover the familial immunoglobulin abnormalities are not restricted to Ig M In this family study we have found no Ig G or Ig A type "M components" but this has been observed by Kalff and Hijmans (12) in a similar systematic family survey and amongst the recently reported cases with familial incidence of essential M components Ig G and Ig M types occurred within a same family (9) The variability of immunoglobulin abnormalities may indicate either that there is more than a single gene abnormality or that we are dealing with a basic disturbance of the homeostatic control of immunoglobulin synthesis and/or immune response

A few observations of familial occurrence of multiple myeloma (13 14 15 16 17 18) and cryoglobulinaemia (19 20) have been published The importance of both genetic and environmental factors in the mice plasma cell tumors is well recognized The abnormalities observed in the families of patients with so called "acquired" primary agammaglobulinaemia (21 22 23 24 25) are very similar to our findings in W M families and lymphoreticular proliferation in some instances of malignant nature does occur in these patients The hypothesis of a close analogy between "acquired" agammaglobulinaemias and macroglobulinaemias could therefore be suggested

References

1 Massari R Fine J M & Metais R *Nature 196* 1 6 1962

2 Seligmann M & Badin J *Rev Fr Et Clin Biol* 7 1107 1962

3 Seligmann, M Danon F & Mihaesco C in γ Globulins *Series Haematologica*, 4 50, Munksgaard Ed, 1965
4 Botturi C Ferrari, S & Veiga A A *Lancet 1* 1171, 1961
5 Vannotti, A *Schweiz med Wschr 93* 1744 1963
6 Coste F Massias P & Menkes C *Rev Rhum 31* 37, 1964
7 Seligmann M Danon F & Fine, J M *Proc Soc Exp Biol Med 114* 482 1963
8 Mancini, G Carbonara A & Heremans J F *Immunochemistry 2* 235 1965
9 Axelsson U & Hällen J *Lancet 2* 369 1965
10 Hallen J *Acta Medica Scandinavica 173* 737 1963
11 Waldenstrom J *Acta Medica Scandinavica 176* 345 1964
12 Kalff M W & Hijmans W Personal communication 1965
13 Mandema E & Wildewanck L S *J Genét Hum 3* 170 1954
14 Nadeau L A, Magalini S I & Stefanini, M *Arch Path 61* 101 1956
15 Herrell, W E, Ruff J D & Bayrd E D *J amer med Ass 167* 1485 1958
16 Hirsch W & Schwarz G *Med Klin 54* 1624 1959
17 Manson K J *Scot med J 6* 183 1961
18 Leoncini D L & Korngold L *Cancer 17* 733 1964
19 Biro, I *Orv Hetil 103* 1709 1962
20 Sitomer G Blum J J & Slavin R E *Amer J Med 34* 565 1963
21 Fudenberg H, German J L & Kunkel H G *Arthr and Rheum 5* 565 1962
22 Wolf J K Gokcen M & Good R A *J Lab Clin Med 61* 230 1963
23 Wollheim F A Belfrage S Coster C Lindholm H *Acta Medica Scandinavica 176* 1 1964
24 Burtin P Buffe D & Grabar P *Ann Inst Pasteur 106* 519 1964
25 Wollheim F A Williams R C *J Lab Clin Med 66* 433 1965

From the Department of Medicine of the University of Basle.

Paraproteinaemia, Bence Jones Proteinuria and Amyloidosis

A clinical Study

By E. Studer-Wobmann and F. Koller

Some years ago at the Congress of the German Society of Internal Medicine in Wiesbaden Jan Waldenström pointed out that although diagnostic methods become more and more complicated the recognition of macroglobulinaemia might be greatly facilitated by a very simple procedure needing only the naked eye: the Sia test. At present a few relatively simple methods (not as simple as the Sia test!) which can be performed in any hospital laboratory and of which immunoelectrophoresis is the most rewarding allow the paraproteinaemias to be recognized and also differentiated with sufficient accuracy.

In our department an average of 6 paraproteinaemias was recognised yearly between 1960 to 1964. Only one macroglobulinaemia was found (by ultracentrifugation) in this 5 years series. *In 1965 with the introduction of immunoelectrophoresis as a routine procedure* 18 paraproteinaemias were diagnosed including 7 plasmocytomas, 5 macroglobulinaemias, 5 "essential" paraproteinaemias and 1 symptomatic macroglobulinaemia. Although more blood samples than previously were sent to us from outside the hospital in 1965 the increase in frequency is due primarily to improved diagnosis.

A summary of the pertinent findings of our cases is given in Table 1. A few tentative conclusions from this clinical study may be of some interest.

Material and Methods

The following methods were used in each case:

Paper Electrophoresis (staining with amido black B). *Immunoelectrophoresis* [Micromethod according to Scheidegger (32)] of serum and urine, the latter being concentrated by dialysis against polyaethylene glycol up to 1:10 or exceptionally up to 1:100. Antisera: Equine antihuman serum, specific antisera against gamma A-, gamma M- and gamma G-globulins and against Bence Jones Protein Type I (IgK) and II (IgL) (from the Blood Transfusion Service Amsterdam, the Behringwerke Marburg Germany and the Highland Laboratories USA). Semiquantitative determination of immunoglobulins for the detection of a deficiency of antibodies with the Agardiffusion technique according to Ouchterlony (2).

Tab 1 Synopsis of the Findings in 18 Patients with Paraproteinemia

Nr	Age	BSR	TP	PE	M Grad	IE (Serum)	UC	UP	BJ	Sternal Puncture	X ray	Ne	Amyloidosis	β Lipo	Complications
1	68	129/133	9 1		g/% β 3,2	γA	—	Alb (	—	Plasmocytoma	+	—	—	285	Diabetes Rheumat Arthritis
2	66	90/120	6 9		γ 4,6	γG	—	BJ	II	Plasmocytoma	+	+	—	581	—
3	69	150/160	6 5		γ 2,3	γM	MG	Alb α2 γ	I	Lymphoid R C	—	—	—	330	Hemorrhagic Disease Pancytopenia
4	48	160/164	9 1		γ 8,5	γM	MG	BJ	II	Lymphoid R C	—	—	—	148	Hemorrhagic Disease Anemia Antibody deficiency Syndrome
5	76	60/89	6 8		γ 4,4	γG	—	BJ	II	Plasmocytoma	—	—	+	502	Diabetes
6	68	95/116	7 7		γ 4,4	γμ	—	BJ	I	Plasmocytoma	—	(+)	—	496	Pemphigus
7	62	90/110	7 1		γ 4,8	γM	MG	Alb α2 BJ	—	Lymphoid R C	—	(+)	—	—	—
8	67	112/130	10 8		γ 5,4	γM	MG	n	I	Lymphoid R C	—	—	—	—	Epistaxis
9	51	79/111	9 5		γ 5,2	γM	MG	—	—	Lymphoid R C	—	—	—	—	—
10	64	50/70	8 3		β 2,7	γG	—	—	—	normal	—	—	—	—	Pneumonia
11	72	6/22	7 1		γ 4,8	γG	—	—	—	normal	—	+	—	—	Bronchiectasia
12	78	63/79	6 2		γ 0,6	γM	n	—		normal	—	(+)	—	128	Diabetes Cold Agglutination Disease
13	68	123/140	10 6		γ 5,5	γG	—	Alb α γ	—	Plasmocytoma	+	—	—	376	Thrombophlebitis
14	60	2/4	7 0		γ 4,7	γG	—	—		normal	—	—	—	475	Diabetes
15	62	20/44	6 7		γ 4,8	γG	—	Alb β γ	—	pl Hyperplasia	—	—	—	513	Glomerulonephritis
16	49	93/125	9 0		β+γ 3,6	γA	—	—	—	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]

Stern I Functure the *ultracentr fuge tech nique* as en ployed only for confirmal on of the results obta ned by immunoelectropho res s in tl e cases with macroglobulinaemia Tl ere was always agreen ent between the two methods

Results and Discussion

Of the 18 cases with paraproteinaemia *observed in 1965 8 presented the cha* racteristics of gamma G (=gamma SS) 6 of gamma M (—gamma 1 M) 3 of gamma A (=gamma 1 A) and 1 of gamma μ (Bence Jones type) globulin

The sedimentation rate was mar *kedly increased in 14 out of the 18* cases slightly increased in 2 and nor mal in 2 The total protein concentra tion in serum was increased only in 7 patients out of 18 in 2 cases it was even slightly decreased

Examination of the *sternal marrow* allowed the diagnosis of plasmocy *toma to be made in 4 out of 8 cases* with gamma G in 2 of 3 cases with gamma A as well as in the case of gamma μ paraproteinaemia in other words in almost half of the cases (in 9 out of 17) there was no correlation between the immunoelectrophoretic and the morphological finding The cases may be classified as "essential" or cryptogenetic paraproteinaemias Our observations give no evidence that they have in fact to be *considered* as premyeloma (Waldenstrom) but this possibility can by no means be excluded It appears that in the early stages of the development of plasmo cytoma the immunoelectrophoretic finding is more reliable than the re sult of the sternal puncture a state *ment* which will be accepted only with reluctance by classical haemato logy! However several observations point in this direction *(Osserman etc)* — On the other hand we observed one patient with primary amyloidosis (confirmed by biopsy) who developed an unequivocal plasmocytoma (sternal puncture) during our observation and *who did not present* any of the cha racteristics of paraproteinaemia Wetc ker has reported a similar case with plasmocytoma (without amyloidosis)

Therefore we have to admit that paraproteinaemia *(of the gamma G* and A type) may occur without plas mocytoma and that conversely plas *mocytoma may in rare instances exist* without paraproteinaemia

Of the 6 cases with *macroglobulin aemia 5* presented the typical lym phoid reticulum cells in the sternal marrow Here again classical haema tology is inclined to consider marrow morphology as more reliable than the abnormality of immunoglobulins However if the cytological variabi lity is taken into account the immuno-*electrophoretic results appear defini* tely more accurate

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Legend A Age BSR Blood Sed mentat on Rate *(mm in the 1st and 2nd hour)* TP Total Pr te n n Serum gr% PE=Paper Electrophoresis Mgrad M Grad ent in gr% IE Im un Electr pl oresis UC Ultracentr fuge MG Macroglobul ns UP—Urinary Proteins, BJ B nce Jones Pr te n Type I & II Lympho d RC Lympho d Ret culumcells PI Hyper pla a Pla acellularhyperplas a Ne=Nephropathy β L po =β-L poprote ns (normal Values (660 o D

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I e I A Age BSR = Blood Sedimentation Rate (mm in the 1st and 2nd hour) TP — Total Protein in Serum gr% PE Paper Electrophoresis Mgrad = M Gradient in gr% IE = Immuno Electrophoresis UC Ultracentrifuge MC = Macroglobulins UI = Urinary Proteins BJ Bence Jones Protein Type I & II Lymphoid RC Lymphoid Reticulumcells Pl Hyperplasia ... acellular hyperplasia Ne — Nephropathy β Lipo = β Lipoproteins (normal Values ... 660 ...)

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5	76	60/89	6 8		γ 1 7	γG	—	BJ	II	Plasmocytoma	—	—	+	502	Diabetes
6	68	95/116	7 7		γ 1 1	γμ	—	BJ	I	Plasmocytoma	—	(+)	—	496	Pemphigus
7	62	90 110	7 1		γ 4 8	γM	MG	Alb α2 BJ	—	Lymphoid R C	—	(+)	—	—	—
8	67	112/130	10 8		γ 5 1	γM	MG	n	I	Lymphoid R C	—	—	—	—	Epistaxis
9	51	79 111	9 5		γ 3 2	γM	MG	—	—	Lymphoid R C	—	—	—	—	—
10	61	50 70	8 3		β 2,7	γG	—	—	—	normal	—	—	—	—	Pneumonia
11	72	6/22	7 1		γ 1 8	γG	—	—	—	normal	—	+	—	—	Bronchiectasis
12	78	63/79	6 2		γ 0 6	γM	n	—		normal	—	(+)	—	328	Diabetes Cold Agglutination Disease
13	68	123 110	10 6		γ 5,5	γG	—	Alb α γ	—	Plasmocytoma	+	—	—	376	Thrombophlebitis
14	60	2 4	7 0		γ 1 7	γG	—	—		normal	—	—	—	475	Diabetes
15	62	20 11	6 7		γ 1 3	γG	—	Alb β γ	—	pl Hyperplasia	—	—	—	591	[illegible]
16	68	93 12[illegible]	[illegible]		β+γ [illegible]										

Sternal Puncture the *ultracentrifuge technique* was employed only for confirmation of the results obtained by immunoelectrophoresis in the cases with macroglobulinaemia There was always agreement between the two methods

Results and Discussion

Of the 18 cases with paraproteinaemia observed in 1965 8 presented the characteristics of gamma G (=gamma SS) 6 of gamma M (=gamma 1 M) 3 of gamma A (—gamma 1 A) and 1 of gamma μ (Bence Jones type) globulin

The sedimentation rate was markedly increased in 14 out of the 18 cases slightly increased in 2 and normal in 2 The total protein concentration in serum was increased only in 7 patients out of 18 in 2 cases it was even slightly decreased

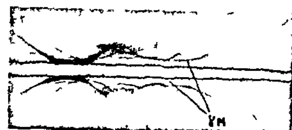
Examination of the *sternal marrow* allowed the diagnosis of plasmocytoma to be made in 4 out of 8 cases with gamma G in 2 of 3 cases with gamma A as well as in the case of gamma μ paraproteinaemia in other words in almost half of the cases (in 5 out of 12) there was no correlation between the immunoelectrophoretic and the morphological finding The cases may be classified as "essential" or "cryptogenetic" paraproteinaemias Our observations give no evidence that they have in fact to be considered as "premyeloma" (Waldenstrom) but this possibility can by no means be excluded It appears that in the early stages of the development of plasmocytoma the immunoelectrophoretic finding is more reliable than the result of the sternal puncture a statement which will be accepted only with reluctance by classical haematology! However several observations point in this direction (Osserman etc) — On the other hand we observed one patient with primary amyloidosis (confirmed by biopsy) who developed an unequivocal plasmocytoma (sternal puncture) during our observation and who did not present any of the characteristics of paraproteinaemia Weicker has reported a similar case with plasmocytoma (without amyloidosis)

Therefore we have to admit that paraproteinaemia (of the gamma G and A type) may occur without plasmocytoma and that conversely plasmocytoma may in rare instances exist without paraproteinaemia

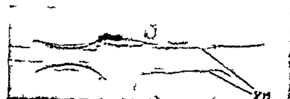
Of the 6 cases with *macroglobulinaemia* 5 presented the typical lymphoid reticulum cells in the sternal marrow Here again classical haematology is inclined to consider marrow morphology as more reliable than the abnormality of immunoglobulins However if the cytological variability is taken into account the immuno electrophoretic results appear definitely more accurate

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Legend A Age BSR Blood Sedimentation Rate (mm in the 1st and 2nd hour) TP=Total Protein in Serum gr%) PE=Paper Electrophoresis Mgrad — M Gradient in gr% IE = Immuno Electrophoresis UC=Ultracentrifuge MG Macroglobulins UP — Urinary Proteins, BJ Bence Jones Protein Type I & II Lymphoid RC — Lymphoid Reticulumcells Pl Hyperplasia Plasmacellularhyperplasia Ne — Nephropathy β-Lipo = β Lipoproteins (normal values to 660 [illegible]



Case 4 Conc 1 3

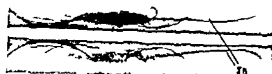


Case 8 Conc 1 3



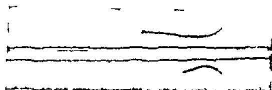
Case 9 Conc 1 3

Fig 1 γM Paraproteinemia Precipitation with equine antihuman serum



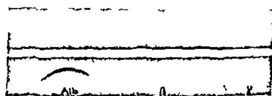
Conc 1 3

Precipitation with antihuman serum from the *horse*



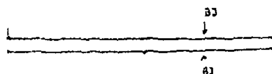
Conc. 1 3

Specific precipitation with anti gamma macro globulin serum pathologic gamma M precipitation line



Conc. 1 3

Identification of the uroproteins with anti human serum Precipitation lines of the albumin (Alb) beta globulin (β) and gamma globulin type (γ)



Conc 1 3

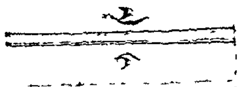
Specific precipitation of the micromolecular uroprotein with anti *BJ* serum type *I*

Fig 2 Gamma M Paraproteinemia M Waldenstrom case 7

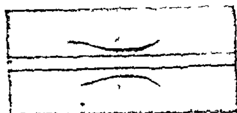
Whereas the quantity of abnormal globulins has apparently no direct prognostic significance in paraproteinaemias in general it seems that the macroglobulinaemias with exceptionally large globulins (which do not migrate in agar gel) present a particularly serious prognosis (cases 3 and 9)

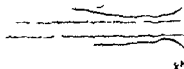
In 1 case macroglobulinaemia was found associated with cold agglutinins

Immunoelectrophoresis being the most reliable method for the detection of *Bence Jones protein* it is of interest to note that with this technique the B J protein may be found in urine and serum of various types of paraproteinaemia including macroglobulinaemia (Table 1 2 cases of gamma G 3 cases of gamma M and the gamma μ case) We confirm therefore the findings of Weicker and others who showed that *Bence Jones protein*



Case 9 2 fractions with immunologically different precipitation the first does not migrate in agar gel





Case 8 2 to 3 various fractions Conc 1 3

Fig 3 Various types of gamma M paraproteinemia (macroglobulinemia) Specific precipitation with equine anti gamma M serum

can occur in all types of paraproteinaemia

However this low molecular protein can — even with immunoelectrophoretic technique — not been detected in all cases of paraproteinaemia (Table 1) Therefore if Kahlers merit of having correlated Bence Jones protein with multiple myeloma subsists it has to be stressed that the absence of this protein rules out plasmocytoma just as little as its presence excludes macroglobulinaemia

The relationship between *amyloidosis* and abnormal gammaglobulins was first suggested by Magnus Levy in 1931 who emphasized the frequent occurrence of Bence Jones protein in this condition Apitz who found abnormal plasma cells in cases of primary amyloidosis designated this disease as "premyeloma" Osserman in a careful study presented evidence that the amyloid infiltrates are formed by aggregates of gammaglobulin fragments of the Bence Jones type with tissue proteins and/or polysaccharides One of our cases with gamma G paraproteinaemia and plasmocytoma presented amyloid deposits in the spleen and liver at autopsy We have already mentioned the case with very pronounced primary amyloidosis (involving the tongue the muscles of the gastrointestinal tract the heart and the kidney) who developed a plasmocytoma during the 9 month of our observation No evidence for paraproteinaemia (or Bence Jones protein) could be found in this patient

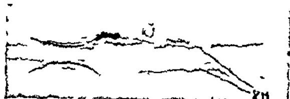
The problem of the development of amyloidosis is far from being solved But the relationship to abnormal immunoglobulins produced by abnormal plasma cells casts some light on the pathogenesis of primary amyloidosis which up to now has been entirely enigmatic

Summary

Immunoelectrophoresis which can be performed in any hospital laboratory



Case 4 Conc. 1 3

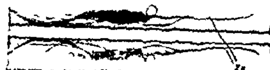


Case 8 Conc 1 3



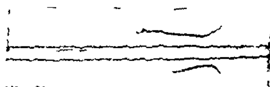
Case 9 *Conc 1 3*

Fig 1 γM Paraproteinemia Precipitation with equine antihuman serum



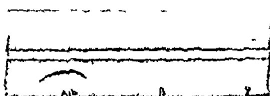
Conc 1 3

Precipitation with antihuman serum from the horse



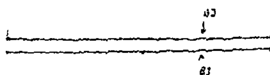
Conc 1 3

Specific precipitation with anti gamma macro globulin serum pathologic gamma M precipi tation line



Conc 1 3

Identification of the uroproteins with anti human serum Precipitation lines of the al bumin (Alb) beta globulin (β) and gamma globulin type (γ)



Conc 1 3

Specific precipitation of the micromolecular uroprotein with anti BJ serum type 1

Fig 2 Gamma M Paraproteinemia (M Wal denstrom) case 7

Whereas the quantity of abnormal globulins has apparently no direct prognostic significance in paraprotein aemias in general, it seems that the macroglobulinaemias with exceptio nally large globulins (which do not migrate in agar gel) present a parti cularly serious prognosis (cases 3 and 9)

In 1 case macroglobulinaemia was found associated with cold agglutinins

Immunoelectrophoresis being the most reliable method for the detection of *Bence Jones protein* it is of interest to note that with this technique the B J protein may be found in urine and serum of various types of para proteinaemia, including macroglobu linaemia (Table 1 2 cases of gamma G 3 cases of gamma M and the gamma μ case) We confirm therefore the findings of Wuicker and others who showed that Bence Jones protein

21 Kahler O Zur Symptomatologie des multiplen Myeloms Beobachtung von Albuminurie Prag med Wschr 14 33 und 45 (1889)

22 Klemm D Hunstein W Harwerth H G (Freiburg) Zur Klassifizierung atypischer paraproteinämischer Hämoblastosen Blut XI 208—235 (1965)

23 Kyle R A Bayrd E D "Primary" Systemic Amyloidosis and Myeloma Arch int Med 107 344—353 (1961)

23 a Magnus Levy A Bence Jones Eiweiss und Amyloid Ztschr klin Med 116 510 (1931)

24 Marki H H u F Wuhrmann Rudimentäre Paraproteinämie Eine Frühform des multiplen Myeloms? Schweiz med Wschr 93 1381 (1963)

25 Olmer J Mongin M Muratore R und Demzet D Myélomes macroglobulinémies et dysglobulinémies Masson Paris 1961

26 Osserman, L F and Takatsuki K Plasma cell myeloma gamma globulin synthesis and structure A review of biochemical and clinical data with the description of a newly recognized and related syndrome "H gamma 2 chain (Franklin's) disease [Medicine (Baltimore) 42 385 (1963)]

27 Ouchterlony O Antigen antibody reactions in gels Ark Kemi (Stockh) 26 b Nr 14 (1948

28 Putnam F W Structural relationships among normal human gamma globulin myeloma globulins and Bence Jones proteins Biochim biophys Acta Amst 63 543 1962)

29 Riva G Makroglobulinämie Waldenström Helv med Acta 2 Heft 3 u ? 1958)

30 Roulet D L A Spengler G A Gugler E Butler R Ricci C Riva G und Hassig A Antigenanalytische Untersuchungen an Paraproteinen Helv med Acta 28 1 1961

31 Roulet D L A Spengler G A und Hassig A Immunelektrophoretische Untersuchungen an Paraproteinen Cancer 14 600 1961)

32 Scheidegger J J Sur une micro méthode d'immunoélectrophorese Int Arch Allergy 7 103 (1955)

33 Scheiffarth F Götz H und Lutz H Zur Charakterisierung von Myelomproteinen und Makroglobulinen Acta haemat 34 144 (1965)

34 Walmann T A Fahey J L and McFarlane A S Metabolism of Bence Jones proteins J clin Invest, 43 103 (1964)

35 Waldenström J Beginnende Myelomatose oder essentielle Hyperglobulinämie mit Fibrinogenopenie — Ein neues Syndrom? Acta med Scand 117 216 (1944)

36 Waldenström J Zwei interessante Syndrome mit Hyperglobulinämie (Purpura hyperglobulinaemica und Makroglobulinämie) Schweiz med Wschr 78, 927 (1948)

37 Waldenström, J Die Makroglobulinämien Ergebn inn Med Kinderheilk 9 586 (1958)

38 Waldenström J Makroglobulinämie Triangel 3 ?(2 (1958)

39 Waldenström J Natürliche Einteilung der Myelome und ähnlicher Zustände Verh VIII Kongr Europ Ges Haemat Nr 163 Wien 1961

40 Waldenström J Störungen der Gamma Globulin Synthese Med Klin 57 1 09 (1962)

41 Waldenström J Die Myelomkrankheit — Vorkommen und Verlauf in einer Stadtbevölkerung Med Klin 59 413 (1964)

42 Weicker H und Huhnstock K Die Paraproteine bei Plasmocytomen und Makroglobulinämien Dtsch Arch klin Med 208 389 (1962)

43 Weicker H Brucher H Huhnstock K Meiser J und Plugge H Paraproteinosen klinische biochemische und morphologische Verlaufsuntersuchungen Dtsch Arch klin Med 211 14 (1965)

44 Wuhrmann F und Marki H H Dysproteinämien und Paraproteinämien Basel und Stuttgart Benno Schwabe & Co 1963

is of value in the diagnosis and differentiation of paraproteinaemias in so far as it is probably superior to sternal puncture in the early recognition of plasmocytoma. Immunoelectrophoresis is also the most reliable method for the detection of Bence Jones protein, which may be found in any type of paraproteinaemia. Primary amyloidosis may precede or accompany plasmocytoma.

References

1 Akbarian M, Fenton J: Perforation of *Small Bowel in Amyloidosis*. Arch int Med 114 6 815 (1965)

1a Apitz K: Die Paraproteinosen. Ueber *die Störung des Eiweiss*stoffwechsels bei Plasmocytom. Virchows Arch path Anat 306 631 (1940)

2 Aron L und Vargues R: Les macroglobulinémies. Etude sur 30 cas confirmés *par* ultracentrifugation. Presse Med 70 1071 (1962)

3 Barandun S, Stampfli G, Spengler G A *und Riva G*: Die Klinik des Antikorpermangelsyndroms. Helv med Acta 26 163 *u 203 (1959)*

4 Berggård L and G M Edelman: Normal counterparts to Bence Jones proteins: *free L Poly*peptide chains of human gamma globulin. Proc nat Acad Sci (Wash) 49 330 (1963)

5 Brecher G, Y Tanaka, R A Malmgren and Fahey J L: Morphology and protein synthesis in multiple myeloma and macroglobulinemia. Ann N Y Aca Sci 113 642 (1964)

6 Brucher H: Untersuchungen zur Morphologie der Makroglobulinamie Waldenstrom. Dtsch Arch klin Med 203 414 (1956/57)

7 Burtin P: Etude immuno electrophoretique des proteines du myelome in Grabar P and Burtin P: Analyse immuno electrophoretique. Masson Paris 1960

8 Cohen S: Properties of the peptide chains of normal and pathological human gamma globulins. Biochem J 89 334 (1963)

9 Eijk H G van, C H Monfoort, J J Witte and H G K Westenbrink: Group characteristic difference in amino acid composition between Bence Jones pro*teins of Burtin's* types I and II. Biochim biophys Acta (Amst) 86 410 (1964)

10 Fahey J L: Chromatographic studies of anomalous gamma, beta 2 A and macroglobulins and normal gamma globulins in myeloma and macroglobulinemic sera. J biol Chem 237 440 (1962)

11 Fahey J L: Structural basis for the dif*ferences between* type I and type II human globulin molecules. J Immunol 91 448 (1963)

12 Franklin E C, M Meltzer, F Guggenheim and J Lowenstein: An unusual micro gamma globulin in the serum and urine of a patient. Fed Proc 22 264 (1963)

13 Franklin E C: Heavy chain disease, a new disorder of serum gamma globulins. Report of the first case. Am J Med 37 332 (1964)

14 Gitlin D und Janeway C A: Genetic alterations in plasma proteins of man in Putnam F W: The plasma proteins. Academic Press New York 1960

15 Grabar P et Burtin P: Analyse immuno electrophoretique. Masson Paris 1960

16 Hässig A: Die Antigenstruktur der Paraproteine. Ergeb Mikrobiol 34 185 (1961)

17 Heremans J: Les globulines seriques du système gamma. Leur nature et leur pathologie. Brussel Arscia und Paris Masson & Cie 1961

18 Heremans J F, Laurell A H, Mårtensson F, Heremans M Th, Laurell C B, Sjöquist J and Waldenstrom J: Die Immunelektrophorese und ihre klinische Bedeutung. Ergeb inn Med u Kinderheilk N F 20 169 (1963)

19 Hitzig W H: Die physiologische Entwicklung der "Immunglobuline" (Gamma und Beta 2 Globuline). Helv paediat Acta 12 596 (1957)

20 Isliker H: Zur Chemie der Makroglobulinamie. Helv med Acta 25 41 (1958)



Fig. 1 Bone marrow smear (sternal puncture on admission) Note the typical dense infiltration of the marrow with small lymphoid cells plasma cells and some tissue mast cells The normal marrow elements are scarce

ba ophils 2 % monocytes 9.5 % and lym ph cytes 2 % The erythrocyte count was 4 mill per mm² The erythrocytes were m tly normochromic normocytic there was m anisocytosis

In the urine there was a trace of albumin and there was no Bence Jones proteinuria The blood urea was 34 mg% latextest l tr lyt s normal Leukocyte alkaline phos phatase 11 (normal 70 to 80) Vital capacity 1 litres (normal 2 3) All tests for tubercu l s negative Microscopically and in the sputum culture no tubercle bacilli Mantoux p sit e at a dilution of 1 1000 Sputum exa minati ns f r fungus were negative comple ment r actions for hist plasmosis and crypto sis w re negative

Sternal marrow There was a severe infiltration of the marrow (Fig 1) with small lymphoid reticulum cells Some of these cells had a small border of surrounding protoplasm of bluish aspect There was a distinct increase of the plasmacellular reticulum cells and the *tissue mast cells* *The erythropoesis* was quantitatively diminished and normoblastic There was a normal activity of the granulocytopoesis and there was a normal amount of megacaryocytes

Chest x ray on admission (Fig 2a) Diffuse small miliary infiltrations which are confluent in some parts of the lung The x ray of the pelvis shows slight *diffuse osteoporosis* but no osteolytic foci

Special examinations for paraproteins Sia test positive total protein 6 g% A/G ratio 0.47 alb 31.8 rel. % α_1 glob 5.3 rel. % α_2 globulin 9.4 rel % β globulin 9.7 rel %, φ globulin 29.1 rel. % and γ globulin 14.7 rel. % viscosity 2.50 to 2.85 Immuno electrophoresis (serum) paraproteinemia of the *γ₁M globulin* type Electrophoresis of the 24 hour urine The slight amount of protein present in the urine almost entirely consisted of the γM type In the ultracentrifugation of the serum we *found an abnormal macroglobulin* with a sedimentation constant *S* of 14 Svedberg units quantitatively they made up about 15 % of the total protein

Biopsies

Lymphnode (Biopsy fecit PD Dr R Berchtold Histology Institute of pathology Aarau Dr I P Muhlethaler) The normal structure of the lymphnodes was almost completely *destroyed* *There were still* some nodules which contained small lymphoid cells capsule of the nodes was infiltrated with small lymphoid cells Diagnosis *malignant lymphoma* of the nodular lymphocytic type

The *liver biopsy* showed a normal histological picture

Lung biopsy (operation by PD Dr R Berchtold) A piece of 3/2.0 8 cm gray brownish lung was excised Histology (Institute of pathology Aarau, Dr I P Muhlethaler) In

From the Medical Department (Head Sven Moeschlin MD)
Bürgerspital Solothurn (Switzerland)

Macroglobulinemia Waldenstrom with miliary lunginfiltrations and terminal plasmacell-leukemia [1]

(Chlorambucil induced clinical remission despite autoradiographically persistant DNA- and RNA synthesis)

By SVEN MOESCHLIN

Introduction My dear friend Jan Waldenstrom has described several new syndroms in the field of internal medicine Despite the modern tendency of overspecialisation, he always remained the big master in both the wide field of general internal medicine and hematology as well Because of his special interest in hematology I would like report a case of a syndrome in this field which bears his own name We have partly described this case before (1), but its further clinical course and the terminal transition into plasmacell leukemia is of a special interest

Case history G E 79 year old woman admitted to the hospital on March 2 1963 with generalized adenopathy and miliary lunginfiltrations with severe cachexia

The disease started in the beginning of 1961 with increasing fatigue and general weakness In summer 1961 an enlarged submandibular lymphnode was noted on the left side since then there was continuous anorexia often nausea and emesis and progressive loss of weight totally 13 5 kg In July 1962 generalized lymphadenopathy was noted and for the first time a miliary lung picture was seen The sedimentation rate was increased to 62 mm in the first hour Histologically an excised submandibular node showed a picture resembling a follicular lymphoblastoma of moderate cell size Since the end of 1962 there was recurrent epistaxis dyspnea cough and progressive cachexia

Clinical findings On the day of admission (March 2 1963) she was thin and pale and had a generalized lymphadenopathy Some of the nodes had the size of a medium plum and they were found in the submandibular cervical supraclavicular axillar and inguinal area The liver was not enlarged the spleen measured 10 1/ cm but was not palpable On auscultation of the lungs and the heart there were no abnormal findings The blood pressure was 140/66 the pulse 96 No infiltrations in the ocular fundus

Laboratory findings on admission Sedimentation rate 130/135 mm (20° C) and 129 134 mm (37° C) Hemoglobin 12 4 g% hematocrit 38% reticulocytes 6‰ thrombocytes 500 000 leukocytes 4 800 with the following differential Juvenile neutrophils 4% segmented neutrophils 56% eosinophils 1 5%

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[1] This study was supported by a grant of the Swiss National Foundation for Scientific Research (No 3587)



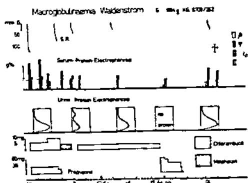
F j 3 Lun„ b յ sy

a T ț cal n l ar no lules n the lungs

l Γ lar en ent 40 Tl ere are d st nct nall nf l rat ons n tl e per l roncl al an l ț r a cular t ue

Enlar n ent 160 The f ltrat ons con of l țl o d ells an l ț la n a cells wl l n ake up the t p cal ț c ure of Wald n t m s l ea e

l l o o„raț l In tute of ț atl olo h ț al Aarau l atholo t n h ef Dr F l Mut le l ale Sl de NB 3o30 63



F g 4 reț resents the years cl n cal cour e of a case of Waldenstroms d sease w th marked ț araț rote nem a paraprote nur a and spec f c lun„ nf l rat ons Af er two years of cont nuous treat ent w th mall doses of Chlorambuc l there was cons derable m ț ro en ent w th con ț l te d aț pearance of the paraț rote nem a of the γ1M tyț e as well as of tl e ț rote nur a The mmuno electro ț hores s of the plasma and ur ne was stron„ly ț o t e before an l turı e l con pletely ne„a t ve after therapy After topp n„ the tre t ment n February 19Co tl ou„h a sl „ht pro te nur a re occurre l dur n„ sun mer and fall and the pat nt d e l of acute pla ma cell leukem a n September 1o6o Two carc non as ›ccu red dur n„ tl e t me f ol erval on One was of tl e p nocellular t pe on the l ț and was cured w l x ray therapy The other was an a lenocarc noma of the cecum and was let ct d at autopsy Tl s case s the only one wl ere tl e spec f c cellular nf ltrat ons of Waldenstroms d ea e coul l be er f ed by b oț sy n the lun„ wh le tl e ț at ent was al e

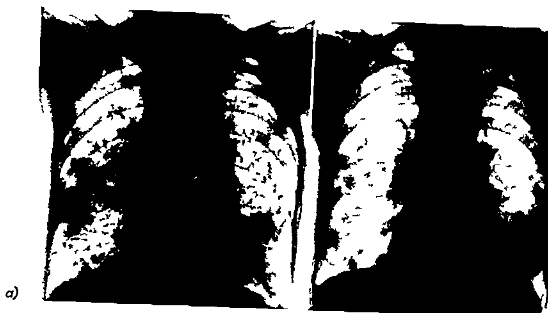
l l d l Sl nt nu l to ga n w gl t l as fe ng y ell Tl e ed e tat on a wa n ț let no n al l Apr l 1 6o 9 a f r d n al from he hosț tal l ere a r ț l le el p e t of a s all ca c r n u ț e l ț an l l e was aga l osț t l z l fr l e May 6 to June 6 19Co A l ț f t s o w s p forn ed and t ț r l l e n are no a sț nocellulare lt l al l conț l l af r rad at on tl erapy a l tl re a o e lence of metastases

F n l nȷs on tl e second hosp t l a lm ss on May 6 1ȷ6ł

Sternal marrow s ll sl owed a ery n arked lympl o d and plasn o ellular f ltrat on, but not so ț ronoun ed as 2 years ago D agnos s ț art al bone n arrow rem ssion of Walden ron s d ea e after Cl loraml u l trea ment.

l u nn y x rays showed d s nct re„res s on of l e puln o ary m l ary nf ltrat ons

Labor tory f l nȷs Sed n entat on rate 12/



a)

Fig 2 a) Chest x ray on admission March 1963 Note the diffuse miliary infiltrations w are confluent in some parts of the lung
b) Striking improvement of the lung picture after two years of treatment with C ambucil 6—10 mg daily combined with prednisone 30 mg daily

teralveolar and especially interlobular septa as well as the peribronchial and perivascular *fibrous tissue* was thickened and infiltrated with cells These infiltrations consisted of lymphocytes and plasma cells and often also of small cells without cytoplasm These cells were also seen intravascularly The cell elements seen in this histological picture resembled the cells seen in Waldenstrom s disease (Fig 3)

Therapy and course Before the lung biopsy had been performed treatment with different antibiotics and tuberculostatics showed no result The lung infiltrations persisted and even increased After cytostatic therapy was begun with Chlorambucil (Leukeran) with an initial dosis of 6 mg daily and later 10 mg daily combined with prednisone 30 mg daily (Fig 4) there was considerable improvement There was a weight gain of 6 kg The general adenopathy decreased and the paraproteinemia slowly returned to normal After some 2¹/ months of therapy on May 27 1963 no paraproteins could be detected in the serum by paper electrophoresis and immuno electro phoresis The improvement of the lung in filtrations and the sternal marrow pic however was much slower Also there w persistent paraproteinuria which was still sent after almost 4 months of treatment

Autoradiographic studies of DNA and the RNA-synthesis Metho Aspirated marrow was incubated o hour in siliconised tubes at 37 with H^3 thymidine H^3 uridine and H cytidine (Further details see (1) These studies showed that the H^3 thymidine (DNA) was localized in th nucleus The same held true for H^3 uridine and H^3 cytidine after 1 hou of incubation Only after an incuba tion of over 2 hours H^3 uridine and H^3 cytosine which are both incorpo rated into RNA had migrated from the nucleus into the cytoplasm

Further course (Fig 4) The patient was discharged from the hospital on July 2 1963 At home she continued to take 4 mg of Chlor

after s opp ng cytostat c treatment The pa t ent was controlled on an ambulatory bas s Sl e rema ned well unt l September 13 1965 Afterwards the general cond t on markedly deter orated There were d st nct ndolent and enlarged lymph nodes n tl e mand bular sul mand l ular cerv cal and ax llar area The l er was markedly enlarged and cm be ow tl e r ght costal margin The spleen was pal pable 3 cm under the left costal marg n Tl ere were petect ae on the forearms and the conjun t ae Body we ght 49 kg

Reexam nat ons of the s gns of Walden s ron s d sease

a *Ste nal marro v* Now aga n tl ere was a d st nct ncrease of young sma l lympho d cells and of plasmocellular ret culumcells Some of the plasmacells had d st nct nuc leol n the r large nucleus

b *Lung p cture* Tl e inf ltrations of the ung ha e ncreased aga n and there are s gns of pleur t s on both s des

Laborato y f nd ngs Sed mentat on rate 14 39 mm hemoglob n 7 a g% leukocytes 2 600 per mm³ w h normal d fferent al ount, thrombo ytes 20 000 per n m³ Pa per electropho e s and mmuno electro phores s of the serum hypoprote nem a n r ased gamn a glob l n Tl e nterpreta t on of tl e gan ma M mmunoglol ul n pre p ta on l ne was d ff cult and s not d s t ct enough to d agnose a gamma M m munog ol ul n parapro e n m a Ur ne lectr p lore s parap ote nur a w th a la ge M g a l ent n l e $\beta-\gamma$ globul n re g Tl s was essent ally l e san e f nd ng as n the ear 1964 The mmuno ec rop l ore s of tl e ur ne ga e no d s n t f n l ng but looks susp c ous for a pa apr te nur a of l e Ben e Jones t pe Ul racen fugat on of the serum In con s o tl e pre ous f nd ngs in 1964 no l n t na roglol ul n l ne s present

Tw t s af er l e last an bula t control t pat nt reenters the hosp tal on September 1 ... a an emergency ca e Tl ere was a e l e rrhag c d athe s w tl ntest al l em rrl age and p fuse b eed ng fro l e n ou h al hougl the pat ent l ad been off Chlorambuc l s nce February 1965) Tl e he moglob n was 1 g% the leukocyte count 1 900 per mm³ prothromb n act v ty (Qu ck) 20% Urea 184 mg% The pat ent d ed desp te of blood transfus ons Epsamone Cort sone ntra enously etc

Blood smear In the d fferent al leukocyte count tl ere were 35 % plasma cells of dis t nct patholo cal aspect (see F g 5 a) The e cells exh b ted a very pronounced polym or ph sm There were sn aller forms and very large forms but all these plasma cells sl ow very large and somet mes up to 2—3 nucleo l n a large nucleus The cell type is very s mi lar to tl e blastforms of the patholog cal plasma cells as seen in other cases of plasma cell leukem a

Sternal marrow The marrow was now comple ely nf trated w th the same poly morph c und fferentiated b g plasma cells w th a f ne chroma nstructure of the nucleus and 2 to 3 very sharp large nucleoli (F g 5 b) The nucleus was very large compared to the small border of deep blue cytoplasm There were very few eos noph l granulocytes and l mpho d ret culum cells left

D i a g n o s s *Term nal acute plasma cell leukem a* w th con plete nf ltrat on of the bone marrow secondary thrombocytopen a w tl se ere *hemorrhages caus ng the death* of the pa ent on September 16 1965

Autopsy (Inst tute of Pathology Kantons sp tal Aarau Dr I P Muhlethaler) Plasmo cellular nf ltrat ons of all lymph nodes the p card um m yocard um and of the lungs w th secondary *amy o dos s* of the lungs Plasmocellular nf trat ons of the spleen l er k dneys and the adrenal se ere nf l ra t on of the *bone marrow* as th s s seen n term nal plasma cell leukem a In ad l t on tl ere was an adenocarc noma of the cecum w th l leed ng into the ntest nal tract and bilateral hypostat c bronchopneumon a

Discussion

This case of Waldenstrom s disease is of special interest from different points of view A woman of 59 years

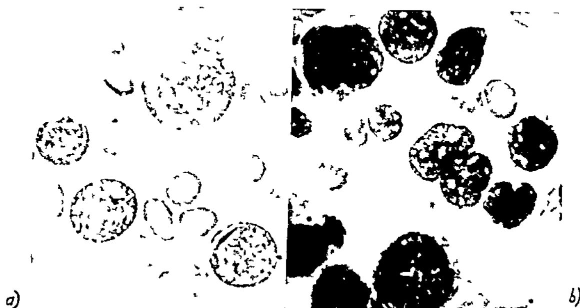


Fig 5 a) Plasma cells of acute plasma cell leukemia in the peripheral blood smear They exhibit a pronounced polymorphism with large nucleoli
b) The sternal marrow was infiltrated with the same polymorphic undifferentiated big plasma cells Only very few marrow elements were left

29, hemoglobin 12 6 g% leukocytes 3 800 with normal differential count thrombocytes 45 000 probably due to the constant cytostatic therapy Serum electrophoresis and immuno electrophoresis no findings whatsoever for a paraproteinemia The sedimentation rate was normal which was in sharp contrast to the one on initial admission Urine electrophoresis trace of protein again there was a small paraprotein gradient in the $\beta-\alpha$ globulin region

The patient was dismissed from the hospital on June 26 1965 Her body weight at that time was 45 kg Therapeutically she continued on 4 mg of Chlorambucil daily (Fig 4) At home she felt well in the beginning In fall 1965 she slowly deteriorated and was again hospitalized on December 23 1965 She remained in hospital until February 18 1965 On this third admission she was febrile and had a bilateral bronchopneumonia The sedimentation rate was again very high (131/141) leukocytes 12 300 with a distinct shift to the left of the neutrophils and toxic granulations After an antibiotic treatment the pneumonic infiltrations disappeared and the general condition improved

Sternal marrow Now completely normal no findings whatsoever of Waldenstrom's disease any more

Pulmonary x ray After disappearance of the bronchopneumonic infiltrations on admission also a further regression of the previous specific infiltrations of Waldenstrom could be detected (see Fig 2 b)

Laboratory findings Sedimentation rate 8 15 hemoglobin 9 g% Urine no albumine leukocytes 3 000 thrombocytes 20—30 000 Serum electrophoresis and immuno electrophoresis no trace of protein anymore Ultracentrifugation no pathological macroglobulin present anymore! (in contrast to the findings in 1963!)

Final course Because of the leukopenia and thrombocytopenia Chlorambucil therapy was stopped in February 1965 Stopping treatment was further justified *by the fact that the electrophoretic and bone marrow findings gave no evidence of active macroglobulinemia of Waldenstrom* Now it was of great interest to see if the previous findings of Waldenstrom s disease would slowly return

urine disappeared completely and the bone marrow picture returned to normal The same normalisation of the serum has been observed by Alkeran treatment in other cases (9 10)

The striking feature of this case is the final transition into an *acute plasma cell leukemia*

It might be possible that the autopsy case described by *Schamaun* (3) also had terminal plasma cell leukemia Here plasma cell infiltrations in nearly all organs were found In our case it was very interesting to note that despite the final recurrence of plasma cell infiltrations of the marrow and the most other organs found at autopsy and despite of the terminal plasma cell leukemia there was only a *slight recurrence of the paraprotein* formation

This in our opinion is probably due to the transition of these cells into *very undifferentiated immature neo*plastic cells in the terminal course of the disease It must be assumed that most of these undifferentiated cells were not able to produce paraproteins any more This can be compared in some way to the cessation of the normal production of hormons in very undifferentiated neoplastic cells for example in a carcinoma of the thyroid etc

The third point which has to be em*phasized in our patient is the occurrence of other malignant lesions* Waldenstrom personally has several times pointed out the strange coincidence of the occurrence of paraproteins and neoplastic transformation *Our patient developed two different carcinomas* first in 1963 a carcinoma of the *upper lip (spinocel*lular type) This lesion could be cured by x ray therapy *At autopsy a second* carcinoma of different type (adeno carcinoma) *of the cecum was found* So this patient developed two different carcinomas *while she had Wal*denström s disease How this neoplastic transformation *is related to Wal*denström s disease is still unknown Is it possible that *the same "virus" once* localizes in the reticulo endothelial *system and other times in other cell* types and causes them to proliferate? *Another possibility is that there is a* direct connection between parapro*teins and malignant transformation* These are only questions which can *be raised but not answered at the* present time

Summary

A case of Waldenstrom s disease with *generalized adenopathy typical bone* marrow infiltration and miliary lung infiltrations which could be proved by biopsy to be specific infiltrations of Waldenström s disease *is reported*

Typical paraproteinemia and albuminuria of the γ1M globulin type were present The patient was treated continously with Chlorambucil during a period of two years and showed a marked improvement The sedimentation rate which was very high initially returned to normal after two years The sternal marrow showed a complete remission with disappearance of the lymphoid and plasmocellular infiltrations and the serum paper and

was suffering from increasing fatigue, anorexia and loss of weight since 1961. In August 1961 she developed generalized progressive adenopathy and in July 1962 a miliary lung picture. Clinically a typical Waldenstrom's disease with a sedimentation rate of 130 mm, a positive Sia test and a paraprotein peak in the γ region on paper electrophoresis of 2.85 g% was found. Immunoelectrophoretically this paraprotein showed to be of the Gamma1M type. On ultracentrifugation it proved to be a macroglobulin of the S 14 Svedberg type and it made up 15 % of the total proteins. Bence Jones proteinuria was not found but there was a distinct paraproteinuria which immunoelectrophoretically showed a distinct M gradient. The sternal marrow was infiltrated with typical lymphoid reticulum cells, plasma cells and tissue mast cells. Fine miliary nodules of the lung proved to be histologically typical lymphoid and plasma cell infiltrations as seen in Waldenstrom's disease infiltrations in the bone marrow. Treatment with Chlorambucil induced an impressive remission during 4 months with a weight gain of 6 kg, normalisation of the sedimentation rate and the serum paper electrophoresis and immuno electrophoresis and nearly complete disappearance of the generalized adenopathy. In the beginning paraproteinuria still persisted and the pathological infiltrations of the bone marrow and the lungs only partially regressed. After further treatment with 4 mg of Chlorambucil daily during one year a complete remission of the bone marrow and a nearly complete remission of the pulmonary infiltrations as well as the adenopathy was induced. In 1964 the serum paper electrophoresis and immuno electrophoresis showed no signs of paraproteinemia any more and finally also the paraproteinuria disappeared. After stopping the Chlorambucil treatment (February 1965) the paraproteinuria reappeared in fall 1965.

In the literature one case of Waldenström's disease with lung infiltrations has been reported "case 8" by *Itere* (2). A more diffuse miliary type has been described for the first time in one case by *Schamaun* (3) in 1954. This was an autopsy finding and clinically the infiltrations had not been detected (4). *Oettgen* et al. (5) reported another case of miliary infiltrations discovered at autopsy. In their case the lung infiltrations had remained clinically undetected (6). *Noach* (7) for the first time in 1956 described a 56 year old patient with Waldenström's disease of the α_2 type with bilateral lung infiltrations. These lesions disappeared after x-ray treatment. Also in the cases of *Revol* (8) and *Itere* (2) (case 2) one year before death a reticular miliary lung picture could be demonstrated as in our case.

In our case the lung infiltrations could be demonstrated for the first time by biopsy in a living patient to be specific infiltrations with plasma cells and lymphoid cells of the Waldenström type. For the first time they also showed distinct regression of a two years continuous treatment with Chlorambucil. At the same time the paraproteins in the serum and in the

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What is Waldenstrom's Macroglobulinemia?

By William Dameshek M D

Waldenström's macroglobulinemia may be said to be characterized chiefly by two features (1) a striking increase in γM globulin of the "monoclonal gammopathy" type and (2) an abnormal bone marrow characterized by either a leukemic or leukemic like picture in which lymphocytes of various types are prominent (1 2 3 4) All the other features — hemorrhagic hemolytic rheologic (viscosity syndrome) must be considered as secondary manifestations Is this condition to be considered a dysproteinemia i e a peculiar disturbance of protein production a form of chronic leukemia in which one of the immunoglobulins is produced in high concentrations or could it conceivably be an abnormal or aberrant (neoplastic) type of immunological response? Perhaps all of these possibilities are correct in one way or another and it is the purpose of this communication in honor of Jan Waldenström's 60th birthday to attempt a unifying hypothesis To do this it is necessary to turn to some relatively basic considerations which involve the immunoglobulins and the immunocompetent cells (immunocytes), and to some clinical considerations regarding autoimmunity and what I have ventured to call the "immunoproliferative disorders" (5 6)

The first stage of the immune response is marked by the production of antibody of the macroglobulin variety following which antibody of the γG type appears (7 8) It is of interest in *this* regard that phylogenetically, γM is the primordial immunoglobulin and γG a later development (9, 10 11) It is conceivable that the γM and the γG *responses* are quite separate although *sequential* and related through a *"feedback"* mechanism (cf below) Parallel studies of tissues during the immune response have indicated that *the* γG phase of the response is associated with plasmocytosis the earlier cellular response to antigen has not

These studies were aided with funds from U S Public Health Service grant number CA 04168 07 from the National Cancer Institute

immuno-electrophoresis showed no findings whatsoever for a paraproteinemia anymore The pathological macroglobulin found by ultracentrifugation had also disappeared after treatment In contrast to the typical findings in 1963, the lung infiltrations were considerably decreased after therapy, but the chest x ray did not return completely to normal Autoradiographic studies of the DNA- and RNA-synthesis by incubation with H^3 thymidine, H^3-uridine and H^3 cytidine still showed active DNA synthesis in the nucleus after one year of treatment Only after incubation of at least two hours, H^3-uridine and H^3 cytidine had migrated from a nucleus into the cytoplasm

In the final course, the patient developed a typical plasma cell leukemia with 35 % plasma cell in the peripheral blood The leukocyte count was 1,900 per mm^3 at that time and all the organs were infiltrated with large polymorphic and undifferentiated plasma cells

These findings were verified by autopsy and in addition a secondary amyloidosis of the lung was found

The patient developed two different carcinomas during the course of her illness One of them was on the upper lip, and was cured with x ray treatment At autopsy, an additional carcinoma of different type, namely an adenocarcinoma of the cecum was found

The different interesting aspects of this case are discussed

References

1 Schmid J R and Moeschlin S Mediz Klinik *59* (1964) 401
2 Fiere H Presse med *65* (1957) 2027
3 Schamaun M Z allg Path *17* (1954) 25
4 Schaub F Schweiz med Wschr *84* (1954) 890
5 *Oettgen H F and Quitmann K Frankf Z Path 67 (1956)* 599
6 Kanzow U Klin Wschr *32* (1954) 154
7 Noach A S Ned T Geneesk *100* (1956) 3881
8 Revol L Creyssel R Morel P and Gauthier Lyon med *196* (1956) 533
9 Waldenstrom J Brit med J II (1964) 859
10 Moeschlin S and Jubin E *Praxis 53* (1964) 1471

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The first stage of the immune response is marked by the production of antibody of the macroglobulin variety following which antibody of the γG type appears (7 8) It is of interest in this regard that phylogenetically, γM is the primordial immunoglobulin and γG a later development (9 10 11) It is conceivable that the γM and the γG responses are quite separate although sequential and related through a "feedback" mechanism (cf below) Parallel studies of tissues during the immune response have indicated that the γG phase of the response is associated with plasmocytosis the earlier cellular response to antigen has not

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yet been clearly defined. It is clear, however, that foreign or "not-self" materials are phagocytized by macrophages (histiocytes, reticuloendothelial cells, probably the most primitive of the protective devices against foreign materials. These phagocytic cells probably digest and "simplify" the engulfed material so that its contained antigen or even a simpler substance *with antigenic information can be* brought into contact with cells more immediately concerned with the cellular immune response (12). It has been shown that small or "mature" lymphocytes *gather about macrophages* containing antigen, sending out pseudopods which may have intimate and prolonged contact with the macrophages (13, 14). Conceivably, the transfer of antigen or of simplified "information" to lymphocytes is made at this time, whence the actual induction of immunization may be said to begin. The lymphocytes then appear to "dedifferentiate" to become large primitive looking cells with pyroninophilic cytoplasm, and variously called blast cells, hemocytoblasts, or, in my own vocabulary, "immunoblasts" (15). These primitive cells may divide and *produce more lymphocytes. Whether* they also produce plasmocytes is not completely certain although it seems very likely. Since the plasmocytes are certainly productive of γG and A globulins, and are not clearly involved in γM production, it would seem likely that the lymphocytes and perhaps the immunoblasts — which contain very large numbers of ribosomes and a primitive ergastoplasm — may be macroglobulin producing units. However, attempts to define this by such techniques as immunofluorescence, cell cultures, etc., have for the most part been unsuccessful. Thus, the exact source of the macroglobulins remains ill defined. Not so with the 7 S globulin, which is clearly associated with the plasma cells (16, 17, 18). The exact derivation of the latter cells, *whether from reticulum cells, lymphocytes*, or from immunoblasts, remains at this time more obscure than ever; it is even possible that the plasmocyte is simply a modified lymphocyte in which the *message for γG protein* (globulin) production has been received and is being acted upon, as indicated by the characteristic ergastoplasmic structure.

It has been demonstrated by Schwartz et al. that the immune response in the experimental animal can be abrogated by a sufficient dosage of the antimetabolite 6 mercaptopurine or other related materials (19). Appropriate manipulation of the drug results in the elimination of the γG response to antigen while the γM response continues unabated. This may be appropriately termed a "block" in *γG prodution and a simultaneous* "locking" of immunoglobulin formation in the γM position. Curiously however, the passive introduction of a small quantity of specific γG (derived from the antibody produced by the same antigen in another animal) results in a rapid fall in γM and then a rise in γG. Thus this experimentally induced "lock" and "block" are at least, in this instance, reversible and

apparently the end result of a disturbance in a normal "feedback" mechanism This seems to be dependent upon the presence of a certain amount of specific 7S when this is supplied either actively or passively the immune response proceeds in its usual fashion

An increase in specific macroglobulin is a normal development in the humoral antibody respone more specifically in its first phase Some infections chiefly of the viral type result in a striking increase in macroglobulin as in primary atypical pneumonia in which cold hemagglutinins are common This increase is either due to a specific and marked reaction to the antigen involved or possibly to the development of a specific group of cells concerned with γM production In infectious mononucleosis a variety of macroglobulins may develop hetero phile agglutinin "syphilitic" antibody cryoglobulin and cold hemagglutinin The extraordinary abnormal lymphocytosis of this condition, the presence of deep blue staining lymphocytes showing marked RNA production and having many of the morphologic characteristics of immunoblasts suggest the possible development of groups of abnormal immunocytes in response to a presumed virus In certain cases of this disease autoimmune reactions develop i e hemolytic anemia or thrombocytopenic purpura this brings up the possibility that a reaction of normal cell antigens with abnormal immunocytes might eventuate in the autoimmune disorders found This we have postulated for various autoimmune disorders

In certain cases of primary atypical pneumonia the concentration of cold hemagglutinins becomes unusually high Successive attacks of this disorder may develop and this may be associated with an extraordinary rise in the titer of cold hemagglutinin to levels of 1 1 000 000 or more If this occurs an immunohemolytic anemia may develop Cold hemagglutinin disease a chronic disorder may be the culmination of repeated attacks of primary atypical pneumonia or other viral disorders which have resulted in the development of an increasingly high titer of cold hemagglutinin (CH) associated with a mild hemolytic anemia and a variably positive Coombs test That the antibody is directed against the I red cell antigen (a self antigen) is strongly indicative of its autoimmune nature The antibody nature of CH is clear that it is also a macroglobulin is also evident However even with titers of CH as high as 1 0 million there may be no indication of an increase in immunoglobulin on the electrophorectic strip On the other hand the continued study of such cases over a period of years may eventually demonstrate one or both of the following a "spike" in the γG area and either a blood or bone marrow lymphocytosis (or both) In other words what is at first "cold hemagglutinin disease" may in some cases eventuate in characteristic "macroglobulinemia" or in chronic lymphocytic leukemia or in both these disorders

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apparently the end result of a disturbance in a normal "feedback" mechanism This seems to be dependent upon the presence of a certain amount of specific 7 S when this is supplied either actively or passively, the immune response proceeds in its usual fashion

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In certain cases of primary atypical pneumonia the concentration of *cold* hemagglutinins becomes unusually high Successive attacks of this disorder may develop and this may be associated with an extraordinary rise in the titer of cold hemagglutinin to levels of 1 1 000 000 or more If this occurs an immunohemolytic anemia may develop Cold hemagglutinin disease, a chronic disorder, may be the culmination of repeated attacks of primary atypical pneumonia, or other viral disorders which have resulted in the development of an increasingly high titer of cold hemagglutinin (CH) associated with a mild hemolytic anemia and a variably positive Coombs test That the antibody is directed against the I red cell antigen (a self antigen) is strongly indicative of its autoimmune nature The antibody nature of CH is clear that it is also a macroglobulin is also evident However even with titers of CH as high as 1 0 million there may be no indication of an increase in immunoglobulin on the electrophoreetic strip On the other hand the continued study of such *cases over a period of* years may eventually demonstrate one or both of the following a "spike" in the γG area and either a blood or bone marrow lymphocytosis (or both) In other words what is at first "cold hemagglutinin disease may in some cases eventuate in characteristic "macroglobulinemia" or in chronic lymphocytic leukemia or in both these disorders

The development of macroglobuli

nemia in the course of apparently typical cold hemagglutinin disease is of considerably theoretical interest Here, a presumably benign immunologic disorder, in which the cold hemagglutinin appears to be an autoantibody and is responsible for the mild hemolytic anemia and the abnormal reactions to cold, develops into (or is followed by) the apparently malignant disorder known as macroglobulinemia It seems clear that cold hemagglutinin is a macroglobulin, perhaps identical with the macroglobulin of macroglobulinemia, which is now present in sufficient concentration to elicit a "spike" in the γG area of the electrophoretic pattern Should these conditions of cold hemagglutinin disease and of macroglobulinemia be considered as separate diseases or are they simply different expressions of the same fundamental disorder?

My own predilections in this matter are based on both the inferences derived from clinical "experiments of nature" and experimental observations both indicate a close relationship and even, at times an actual identity between certain autoimmune disturbances and certain kinds of neoplastic processes of the immunocytes that I have called immunoproliferative disorders" Thus, not only do many cases of chronic lymphocytic leukemia and lymphosarcomatosis show various features of autoimmunity during their course, but there are cases of autoimmune disease — hemolytic anemia thyroiditis Sjögren disease systemic lupus — which may eventuate in various forms of lymphoproliferative disease (20, 21) New Zealand black mice with autoimmune hemolytic anemia often develop (10 percent) a generalized "lymphomatous" disorder (22) Aleutian mink with a diffuse hypergammaglobulinemia and various immunologic disorders may develop (10 percent) the typical clinical and laboratory features of multiple myeloma with a γ globulin "spike (23) The graft vs host disease (runt disease, homologous disease) of the experimental mouse is often followed — in the recovered animal — by a generalized neoplasia of the lymphoid tissue (24) In all these instances, an abnormal immunologic disorder eventually becomes a neoplasia of the leukemic variety, i e, an immunoproliferative disorder

The "immunoproliferative" disorders, as I think of them, are abnormal, usually neoplastic disturbances of the cells concerned with the immune mechanism, i e, the immunocytes comprising lymphocytes plasmocytes, and the reticuloendothelial cells I have included amongst these disorders chronic lymphocytic leukemia generalized lymphosarcomatosis macroglobulinemia and "heavy chain" disease all of lymphoid origin multiple myeloma — plasmocytic, Hodgkin s disease certain forms of reticulosis and possibly sarcoid, of reticuloendothelial cell origin In view of the immune potentiality of the cells concerned in these neoplastic processes it should not be considered strange that immunologic aberrations are almost always present in these conditions Thus autoimmune disturbances as well as various forms

of dysgammaglobulinemia (hypo gamma hypergamma "spikes" of γG — rare — or of γM globulin) are common in the lymphoproliferative disorders In multiple myeloma "monoclonal gammopathy" is the rule except in the "light chain" variety in which only Bence Jones urinary proteinuria is found In Hodgkin's disease and sarcoidosis anergy is almost always present

Autoimmunity may have several pathogenetic mechanisms in my own thinking the Burnetian concept of the "forbidden clone" has loomed large 25 26 27) This is based largely on the inferences drawn from from clinical data both human and animal and on the experimental data already discussed It may be hypothesized that a "forbidden clone" of immunocytes is composed of cells of a special cell line having the capacity to react with a specific self antigen The "forbidden clone" may therefore be thought of as an abnormal "subversive" or foreign" group of immunocytes of identical genetic configuration which may conceivably live in tolerance with the host for some time but which may on the other hand become of clinical importance if tolerance is lost It may either be genetically derived or transplacentally transmitted produced by an infection or continuously fed" by continued infection or it may be the endresult of a non lethal mutation Possibly some abnormal clones may have their origin in the thymus In any event the hypothetical clone may at first result in no apparent effects later with increasing size an autoantibody may be demonstrated (cold hemagglutinin antithyroid antibody rheumatoid factor etc) still later, autoimmune disease may be present — presumably because the clone has expanded sufficiently so that selfantigens reacting against it will result in harmful self or autoantibodies Eventually in some cases expansion of the presumed clone may proceed to such a degree that what we call leukemia or "lymphoma" — i e, a gross, generalized tumor — develops with all its various features The disorder of macroglobulinemia may represent such a condition

From the very first studies of macroglobulinemia as made by Waldenström (1944) the bone marrow was found to contain a preponderance of lymphocytes with plasma cells either low normal or perhaps slightly increased Thus although the condition had many resemblances to multiple myeloma the main difference at least from the standpoint of the marrow lay in the lymphocytosis as opposed to the morphology of multiple myeloma i e plasmocytosis Although the lymphocytosis in the marrow is usually striking there has apparently been a reluctance to call the cells concerned typical lymphocytes or to designate the condition present as lymphocytic leukemia even in the presence of the characteristic blood picture of that disease Thus such names as "reticulo lymphocytosis" "mixed up" lymphocytosis "para lymphocytes" "lymphocytes with naked nuclei" lymphoid reticulum cells etc have been used for the morphologic

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picture in the marrow Our own studies indicate that the preponderant cells present have all the morphological features of lymphocytes, that the lymphocyte morphology varies from case to case or in the same case, i e, from small to medium to large lymphocytes with a varying number of reticulum cells and "immunoblasts" To be sure, "atypical" lymphocytes are often present, as they are in some cases of chronic lymphocytic leukemia or lymphosarcoma Plasma cells in low or normal values have been described

It is possible to infer that the abnormal lymphocytosis of the bone marrow is related to the macroglobulinemia, just as in multiple myeloma the plasmocytosis is related to the γG hyperglobulinemia This would imply that the lymphocytosis is productive of the macroglobulinemia, and again by implication, that the lymphocyte can be productive of macroglobulin Such conclusions from this and other "experiments of nature" have often cast a bright light upon hitherto obscure metabolic abnormalities That macroglobulins are actually produced by lymphocytes is difficult to "nail down", however, although there is much suggestive evidence for this possibility In Waldenström's macroglobulinemia the studies of Zucker Franklin et al using both C^{14} labeled lysine cultures of lymphoid tissues together with immunofluorescent techniques have clearly indicated that medium and large lymphocytes were synthesizing macroglobulin (28) Whether some of the lymphocytes called "large were actually immunoblasts could not be gleaned from this study, but Figure 2 in their paper showing an imprint of a node shows very large primitive appearing cells characteristic of the immunoblast It would be tempting to conclude at this time that, as with γG hyperglobulinemia of multiple myeloma and its plasmocytosis, the γM hyperglobulinemia of the macroglobulinemia is directly related to the lymphoproliferative reaction Since the mature lymphocyte has no visible ergastoplasm and seems otherwise ill adapted to the production of protein, it may well be that the more immature lymphocyte and especially the "de differentiated" lymphocyte or immunoblast with its large complement of ribosomes may be productive of the macroglobulin

The production of macroglobulin the marked lymphocytosis often of an abnormal type, the paucity of plasmocytes and of γG globulin in most cases bring up the question of an acquired abnormality in the normal lymphoid response to antigenic stimulation i e a disorder of the immunproliferative mechanism It is conceivable that an "insult' — whether chemical radiation or viral could delete or otherwise modify a key enzyme involved either in the transformation of lymphocyte to immunoblasts or in the development of the plasma cell (from immunoblast? from lymphocyte?) Either or both of these possibilities could result in an excessive or uninhibited production of 19 S globulin as compared with 7 S production This might be thought of as analogous with the

"locking" of immunoglobulin production in 19 S formation by the use of an immunosuppressive drug with a simultaneous block of 7 S globulin synthesis as already noted (8) It is furthermore conceivable that such an acquired disturbance of the "feedback" shutoff mechanism could become a permanent one and thus react immunologically in a self perpetuating fashion with identical antigens Such an abnormality could eventually become clonal and finally generalized thus resulting in the various laboratory and clinical features of macroglobulinemia Is this disorder then to be considered a neoplastic process, or a disorder of protein metabolism i e a dysgammaglobulinemia? Actually it is both A neoplasm may be defined as a self perpetuating proliferation of a new "race" or clone of cells This clone composed of cells having the identical genetic background may arise in various ways (somatic mutation) viral modification enzyme deletion etc) In order to flourish the clone must possess a certain ecologic advantage thus allowing it to proliferate at the expense of normal cell lines A neoplastic clone of immunocytes being derived from immunologically competent cells could conceivably be subject to antigenic stimulation and thus be productive of immunoglobulins in this instance of the γM variety Initially the macroglobulinemia might be occult i e found only by electrophoretic or other investigation of the serum later it might be present in sufficient concentration to result in cold hemagglutinin disease finally, with continued expansion of the presumed clone, it could result in the disease macroglobulinemia

To return to the question in our introductory paragraph it would seem that in macroglobulinemia we are dealing with an aberrant, self perpetuating disorder of immunoglobulin production with *both immunologic* and leukemic relevance That an abnormality of protein production is present is self evident That this is a neoplastic process based on a possible mutation in which a key enzyme has been lost thus leading to the continued production of macroglobulin is of course speculative but worthwhile pursuing experimentally

Jan Waldenström s many contributions to the "immunoproliferative disorders" even though he may wince at the term — (not that he has not concocted some himself!) — have opened our eyes to the wonders of the "gammopathies" of which he is without question The Compleat Master!

References

1 Waldenstrom J Incipient myelomatosis or essential hyperglobulinemia with fibrinogenopenia Acta Med Scand *117* 216 1944

2 Waldenstrom J Macroglobulinemia Proc VIth Congress of Hematology Copenhagen 1957 Basel 1959 S Karger Vol 2

3 Waldenstrom J Studies on conditions associated with disturbed gamma globuline formation (gammopathies) The Harvey Lectures 211 series 56 1961

4 Waldenstrom, J In PROGRESS IN HEMATOLOGY Grune and Stratton New York Vol II p 266 1962

5 Dameshek W and Gunz, G LEUKEMIA

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L'hétérogénéité des Protéines Myélomateuses

Par R. Creyssel et G. B. Richard
(Lyon, France)

La microhétérogénéité est un caractère fondamental des immunoglobulines normales chez l'homme comme chez les diverses espèces animales étudiées. Toutes les familles d'immunoglobulines actuellement individualisées apparaissent formées d'un spectre continu *de molécules aux structures ioniques* variant graduellement; certaines présentent des degrés divers de polymérisation sans cependant que s'objective un polymorphisme analogue à celui présenté par d'autres protéines sériques (haptoglobines, transferrines) ou par les isoenzymes.

Suivant la conception de Burnet (6) la production des immunoglobulines est le fait de multiples clones cellulaires dont chacun synthétise un type moléculaire strictement défini tant par des capacités fonctionnelles d'anticorps que par sa structure. Lors de l'agression immunitaire, l'organisme sollicité par les multiples structures antigéniques de l'agent pathogène répond par une réaction "polyclonale": il produit une gamme étendue de molécules immunoglobuliniques d'où l'aspect en bande large de l'hypergammaglobulinémie plasmatique. A l'opposé, dans certaines proliférations des cellules immunologiquement compétentes sont produites en abondance, en l'absence d'un stimulus antigénique connu, des immunoglobulines parentes de celles du sujet sain, mais d'homogénéité anormale, cependant que se manifeste en général un déficit de la production des constituants normaux. Il s'agit alors, selon la conception de Waldenström (39, 40) d'une "gammopathie monoclonale" liée à la prolifération de cellules issues vraisemblablement d'un clone unique, mais rien ne permet de décider si ces molécules d'aspect pathologique ont une structure anormale ou si elles sont représentées à l'état de traces dans le sérum du sujet sain.

Mais malgré leur homogénéité physique et structurale frappante, les globulines myélomateuses et les macroglobulines de Waldenström ne sont pas strictement monomorphes. Homogènes quant à leurs caractères antigéniques et génétiques, elles présentent très fréquemment une *hétérogénéité physique systématisée*, véritable poly-

2nd Ed Grune and Stratton New York 1964
6 Dameshek W Immunoproliferative Disorders (Editorial) Bull Internat Union against Cancer *3* 1 1965
7 Bauer, D C Sequences of synthesis of γ *1* macroglobulins and γ 2 globulin antibodies during primary and secondary responses to proteins salmonella antigens and phage J Exp Med *117* 889 1963
8 Sahiar K and Schwartz R S The immunoglobulin sequence J Immunol *95* 345 1965
9 Uhr J W Finkelstein M S and Franklin F C Antibody response to bacteriophage Ø×174 in non mammalian vertebrates Proc Soc Exp Biol & Med *111* 13 1962
10 Grey H M Phylogeny of the immune response J Immunol *91* 819 1963
11 Marchalonis J and Edelman G M Phylogenic origins of antibody structure J Exp Med *122* 601 1965
12 Fishman M Antigenic formation *in vitro* J Exp Med *117* 603 1962
13 Schoenberg M D Numan V R Moore R N and Weisberger A S Cytoplasmic interaction between macrophages and lymphocytic cells in malibody synthesis Science *143* 964 1964
14 McFarland W and Heilman D H Lymphocyte foot appendage its role in lymphocyte function and immunological reactions Nature *205* 887 1965
15 Dameshek W "Immunocytes" and "immunoblasts" — an attempt at a functional nomeclature Blood *21* 243 1963
16 Ortega L C and Mellors R C Cellular sites of formation of gammaglobulin J Exp Med *106* 627 1957
17 White R G Antibody production by single cells Nature *182* 1383 1958
18 Nossal G J V and Lederberg J Antibody production by single cells Nature *181* 1419 1958
19 Sahiar K and Schwartz R S The immunoglobulin sequence Arrest by 6 mercaptopurine and restitution by antibody antigen or splenectomy J Immunol, *95* 345 1965
20 Talal N and Bunim J J The development of malignant lymphoma in the course of Sjogren s syndrome Am J Med *36* 529 1964
21 Rosenbaum M C Pisciotta A V Komninos Z D Goldenberg H and Dameshek W The autoimmune hemolytic anemia of malignant lymphocytic disease Blood *10* 197 1955
22 Mellors R C Autoimmune disease in NZB/Bl mice Autoimmunity and malignant lymphoma Blood in press (1966)
23 Porter D D Dixon F J and Larsen A W The development of a myeloma like condition in mink with Aleutian disease Blood *25* 736 1965
24 Schwartz R S Malignant lymphomas following allogenic disease Transition from an immunological to a neoplastic disorder Science *149* 1511 1965
25 Burnet F M The clonal selection theory of acquired immunity Vanderbilt Univ Press 1959
26 Burnet F M Autoimmune disease Brit Med J 2 645 1959
27 Dameshek W Conceptual advances in immunology and oncology In AUTO IMMUNITY — EXPERIMENTAL AND CLINICAL ASPECTS Harper Hoeber New York p 37 1963
28 Zucker Franklin D Franklin E C and Cooper W S Production of macroglobulins *in vitro* and a study of their cellular origin Blood *20* 56 1962

génique et une composition peptidique semblables (5)

Lorsque la P B J appartient au groupe II de Korngold ("L") elle existe la plupart du temps sinon constamment (Gally et Edelman 19) sous forme de dimère stable du à la formation d un pont disulfure Lorsque par contre la P B J est du type I de Korngold ("K) la polymérisation est essentiellement de caractère non covalent il existe un équilibre monomère — dimère qu objective la dépendance de la constante de sédimentation vis à vis de la concentration protéique

b) — *L Hétérogenéité polymorphique*

A l hétérogenéité montrée en gel d amidon par certaines P B J peut correspondre la présence de constituants de constante de sédimentation identique et d antigénicité commune mais qui montrent de légères différences *de comportement thermique et se* différencient A l étude des cartes peptidiques *par des divergences portant* sur un ou deux peptides Après réduction et alkylation l électrophorèse en gel d amidon en tampon urée formate montre ici l existence de plusieurs bandes (Van Eijk et col 14)

Cette hétérogenéité polymorphique que l on va retrouver dans le cas des immunoglobulines γG myélomateuses *peut ici se superposer à l hétérogenéité* polymérique

I hétérogenéité des globulines myélomateuses γG

C est l électrophorèse en gel d amidon qui a permis d objectiver cette hétérogenéité aussi bien chez l homme (Owen, Got et Silberman 28, Engle et Woods 15 Flynn et Stow 18 Scheurlen 38) que chez la souris porteuse d un plasmocytome transplantable (Askonas 1) Il s agit d un aspect en *multiples bandes* régulièrement disposées (Fig 1 b) dont à pH 8 6 l'importance quantitative décroit avec la mobilité anodique Il ne s agit pas ici d un équilibre de formes métastables Séparées par chromatographie sur cellulose échangeuse d ions (Fahey 16) ou par élution à partir du gel d amidon ces fractions retrouvent leur mobilité et leur rapport quantitatif après nouvelle migration électrophorétique en gel d amidon (Richard 36) Elles ont même comportement en gel filtration, même constante de sédimentation (Putnam et Udin 31) Elles ont une antigénicité commune (Fahey 16 Scheurlen 38)

Il apparait donc s agir d une *hétérogenéité purement électrique* dont l aspect discontinu en bandes s oppose au spectre continu des γG physiologiques elles aussi homogènes au point de vue dimensionnel L étude des subunités de la molécule permet d en *préciser* la nature

Après réduction et alkylation d une *protéine myélomateuse* γG l électrophorèse en gel d amidon en milieu contenant de l urée montre que le fragment "L" (chaine légère) diffère profondément dans son aspect de celui qui provient de gamma globulines normales cependant que le fragment H (chaine lourde) diffère peu de son homologue normal

En tampon glycine urée ou tris citrique urée l électrophorèse en gel d ami

morphisme qui s'oppose a la microheterogeneite de leurs homologues physiologiques Ce caractere doit semble-t il, être rattache selon les cas a l'heterogeneite des structures primaires (γG globulines myelomateuses), ou a l'existence de C polymerisation d une unite structurale de base (γA et γM globulines), il peut participer de l'un et l'autre de ces mecanismes et sa nature reste encore parfois incompletement precisee

Il convient, de dissocier du probleme de l'heterogeneite des proteines myelomateuses celui que pose la co existence, chez le même malade de plusieurs globulines anormales S il est banal de trouver dans les urines, a cote de la proteine de Bence-Jones une proteine myelomateuse d origine seri que, il est par contre rare de voir dans le serum une proteine de Bence-Jones d'abondance suffisante pour donner un pic anormal sur l electrophore gramme bien distinct de celui de la globuline myelomateuse γG ou γA (5 cas sur 480 dans notre statistique per sonnelle — Il peut par ailleurs arriver qu'a la proliferation d une globuline myelomateuse s associe non un deficit de la production des autres immuno globulines, mais une hyperglobulinemie reactionnelle, de type polyclonal dont l importance peut etre suffisante pour faire discuter une double ano malie

L'heterogeneite des proteines de Bence-Jones (P B J)

L'heterogeneite physique des P B J a ete mise en evidence bien avant que fut connue leur signification nosologique, par l'ultracentrifugation (Putnam 32) l'electrophorese en veine liquide (Putnam et Stelos 33), la precipitation par les sels neutres L electrophorese *en gel* d'amidon (Engle et Woods 15 —, *Flynn et Stow 18* —, *Creyssel et Fine* 10), la chromatographie sur cellulose echangeuse d ions (Lynn Phelps et Putnam 26) la gel filtration (Bernier et Putnam 5) ont permis d'objectiver plus nettement l'existence de plusieurs fractions dans les P B J produites chez un meme sujet (Fig 1 a) Si on met a part les fausses heterogeneitees, liees a la presence d autres proteines urinaires ou a la degradation accidentelle de la P B J il semble a la suite des recherches de Bernier et Putnam (5) de Van Eijk, Monfoort et Westenbrink (14) que l'on puisse distinguer pour la P B J deux types d heterogeneitees

a) — *L'heterogeneite polymerique*

La P B J est formee (Edelman et Gally 13) de chaines polypeptidiques legeres "L identiques a celles obtenues a partir des immunoglobulines myelomateuses seriques lors du clivage par reduction Son homologue normal est constitue par les gamma micromoleculaires physiologiques

Elle peut exister sous forme de *monomere* de la chaine L (P M 22 000 environ) plus souvent sous forme de *dimere* (P M 44 000), parfois sous forme de tetramere Isolees par chromatographie sur cellulose echangeuse d ions ou gel filtration (5) separees au cours de la migration en gel d amidon ces composants ont une structure anti

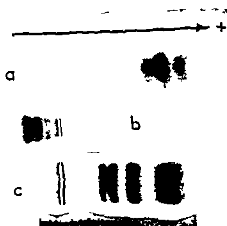


Figure 1 Electrophorese en gel d amidon en tampon borate pH 8 6 de diverses proteines myelomateuses
a) Proteine de Bence Jones
b Proteine myelomateuse γG
c Proteine myelomateuse γA (le diagramme d immunoelectrophorese en gel d amidon est figuré)

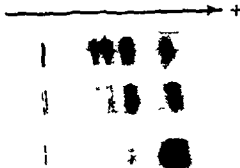


Figure 2 Fractionnement d une proteine myelomateuse γA par gel filtration analyse des fractions successives par electrophorese en gel d amidon)

constituants (Fig 1 c) dont la distribution systematisee evoque l image des haptoglobines 2—2 et 2—1 (Creyssel Manuel Richard et Fine 11) dans d autres cas l heterogeneite est moins nette limitee à l existence de deux fractions elle manque enfin parfois

Une correlation peut etre etablie entre l heterogeneite dimensionnelle et celle que montre la migration en gel d amidon grace a l isolement par *chromatographie* sur cellulose echangeuse d ions (Fahey 16 Creyssel Richard Manuel et Fine 12) ou par *gel filtration* (Richard 35) de diverses fractions des globulines myelomateuses γA (Fig 2) Les constituants dont la migration est la plus retardee en gel d amidon correspondent a ceux qui sont le plus precocement elues lors de la gel filtration et aux constituants lourds definis par l ultracentrifugation (9 11 13 S) a l inverse la fraction qui contient le constituant leger 6 6 S correspond au constituant dont la mobilite anodique est la plus grande dans le gel d amidon

— On peut donc penser que l heterogeneite electrophoretique des proteines myelomateuses γA est essentiellement de type *polymerique* comparable a celle presentee *par certaines* P B J ou par les macroglobulines le gel d amidon par son pouvoir de filtration ne ferait qu objectiver l inhomogeneite dimensionnelle Fahey (16) Levin et col (25) ont montre que sous l action du mercaptoethanol les constituants γA de S_{20} elevé sont dissocies et disparaissent a peu pres completement au profit du constituant 6 6 S Et nous avons pu noter que les fractions "lourdes" separees par gel filtration semblaient apres stockage se transformer partiellement en fractions legeres

don resoud en effet les chaines 'L normales en 10 à 12 constituants regulierement disposes dans le gel (Cohen et Porter 8, Poulik 30) Les chaines L provenant de globulines myelomateuses γG, dans les memes conditions ne donnent par contre qu'un nombre restreint de bandes (6 au maximum pour Cohen 8), bandes de mobilite identique a celles des precedentes et ceci qu il s'agisse de chaines de type Kappa ou lambda (Cohen et Gordon 7)

La chaine L" myelomateuse n est d'ailleurs peut être pas seule en cause dans le genese de l'heterogeneite des globulines γG Migita et Putnam (27) ont montre le caractere heterogene du fragment F papainique provenant des gamma globulines normales et ses variations d'aspect lorsqu'il provient de globulines de myelome ce qu ont confirme plus recemment Poulik et Shuster (31) Askonas et Fahey (2) ont etudie les differences d'aspect des hydrolysats papainiques prepares a partir de fractions separees chromatographiquement dans une globuline myelomateuse γG de souris

Il semble donc qu ici comme dans le cas des P B J — et contrairement a certaines constatations initiales — puissent exister en divers secteurs de la proteine myelomateuse des facteurs d'heterogeneite electrique

L'heterogeneite des proteines myelomateuses γA

— Il existe une *heterogeneite dimensionnelle certaine* des globulines myelomateuses γA, demontree par les donnees de l ultracentrifugation Deja notee par Kekwik (23), elle a ete etudiee par nombre d auteurs (Putnam et Udin 34, Rundles, Cooper et Willet 37 Peterman Hamilton et Korngold 29 Creyssel, Cille, Coulon et Morel 9 Laurell 24) A cote du composant 6,6 S ces proteines comportent des fractions de constante de sedimentation plus elevee, 8, 11, 13 et eventuellement 15 S, dans certains cas la predominance des fragments lourds peut justifier le terme de *macroglobulinemie atypique* employe par Jahnke et Scholtan (22) Les preparations purifiees de γA globulines physiologiquer contiennent d ailleurs (Heremans 21) de petites quantites de ces constituants lourds

La periodicite que presentent les valeurs de S_{20} de ces constituants evoque l existence d'une polymerisation a partir d une molecule monomere de poids moleculaire 150 000 environ comme l'ont confirme recemment Levin Ritzmann Seeuwen et Nanninga (25)

Les proteines myelomateuses γA montrent avec une grande frequence une *inhomogeneite electrophoretique* l'image en "bande etroite" est ici moins nette que dans le cas des myelomes γG et parfois meme s ebauche sa separation en plusieurs pics lors qu on examine ces proteines par electrophorese en veine liquide ou sur support *L electrophorese en gel d amidon* permet une resolution plus poussee dans un grand nombre de cas et ceci surtout lorsque la mobilite de la proteine normale est elevee en veine liquide la migration a travers le gel la fractionne en un certain nombre de

6 Burnet M The clonal selection theory of antibody production — Oxford—London — New York 1959
7 Cohen S Gordon S Biochem J 1965 97 460
8 Cohen S Porter R R Biochem J 1964 90 284
9 Creyssel R Cille Y., Coulon P Morel P Bull Chim Biol 1958 40 469
10 Creyssel R Fine J M Rev Franç Etudes Clin et Biol 1962 VII 748
11 Creyssel, R., Manuel, Y Richard G B Fine J M Rev Franç Etudes Clin Biol 1962 VII 253
12 Creyssel R Richard G B Manuel, Y Fine J M Clin Chim Acta 1962 7 869
13 Edelman G M Gally J A J Exp Med 1962 116 207
14 Eijk H G Van, Monfoort, C. H., Westenbrink H G K Proc Kononkl Neder Akademie Van Wetenshhappen 1963 Séries C 66 345
15 Engle L Wood K R. J Clin Invest. 1957 37 888
16 Fahey J L Jr Clin Invest 1963 42 111
17 Fine J M Boffa G A Creyssel R Clin Chim Acta 1964 9 526
18 Flynn, F V Stow E A J Clin Path 1958 11 338
19 Gally J A Edelman G M Biochem Biophys Acta 1965 94 175
20 Heremans J F Séries Haematologica (supp Scand Jr Haematology) 1965 4 17
21 Heremans J F Les globulines seriques du systeme gamma — Bruxelles Paris 1960
22 Jahnke K Schollan W Verh Dtsch Ges Inn Med (1955) 61 312
23 Kekwick, R. A Biochem. J 1940 34 1248.
24 Laurell, H Acta Med Scand 1961 supp 367 69
25 Levin W C Ritzmann S E., Seeuwens J P., Nanninga L. Clin. Chim. Acta 1964 10 12
26 Lynn L. T., Phelps R. A., Putnam F W Unpublished observations — cite dans (32)
27 Migita S., Putnam F W J Exp Med 1963 117 81
28 Owen, J A., Got, C., Silberman H J Clin Chim Acta 1958, 3 605
29 Peterman, M L Hamilton M G., Korngold L Cancer (Philad) 1956 9 193
30 Poulik M D Nature (Lond) — 1964 202 1174
31 Poulik, M D., Shuster J Nature 1964 204 577
32 Putnam F W The plasma proteins — New York — London 1960 — Tome II
33 Putnam F W., Stelos P J Biol. Chem 1953 203 347
34 Putnam F W Udin, B J Biol Chem 1953 202 727
35 Richard G B Clin Chim Acta, 1965 11 399
36 Richard G B These de Sciences (en preparation)
37 Rundles R W., Cooper G R Willett, R W J Clin Invest 1951 10 1125
38 Scheurlen P G Klin Wschfrt 1963 41 723
39 Waldenstrom J Harvey Lectures 1961 56 211
40 Waldenström, J Progress in Haematology III 1962 266

Mais il existe certains faits cependant qui concordent mal avec la conception d'un simple equilibre de polymerisation

— L'importance quantitative des divers composants de la globuline myelomateuse γA varie d'un cas a l autre, qu'on l apprecie par les donnees de la sedimentation ou par celles de l electrophorese en gel d amidon, il existe un *polymorphisme* de l hetero geneite, que cette derniere technique met particulierement bien en evidence, Ballieux (4), Fine, Boffa et Creyssel (17) et qui a fait evoquer la possibilite d'une regulation genetique

— *L'action des thiols* presente des modalites quant a ses resultats utilisant le thioglycolate de sodium nous avons observe, par electrophorese en gel d amidon que les proteines myelo mateuses γA d heterogeneite accentuee et systematisee n etaient que partiellement transformees, deux constituants nettement distincts restent visibles dans le gel Lorsque l heterogeneite est de caractere moins regulier, on note la reduction a un seul constituant Auscher et Guinand (3) ont pu par ultracentrifugation preparative separer a partir d une globuline myeloma teuse γA cryo precipitable une fraction 6 75 S des constituants plus lourds L action de la cysteine fait disparaitre les composants 11 et 13 S au profit du composant 6 75 S mais le composant 9 S ne semble pas modi fie

— Des *differences dans les cartes peptidiques* des fractions legeres et lourdes provenant de memes globulines myelomateuses γA ont ete notees par Ballieux (4)

Il existe d'ailleurs, ici comme dans le cas des proteines myelomateuses γG une *heterogeneite des sub unites* pro duites par reduction ou par hydrolyse enzymatique l'heterogeneite des chai nes L (Poulik) (30) et des fragments F papainiques (Poulik et Shuster) (31) presente une image differente de celle trouvee pour les globulines γA nor males

Ces faits permettent de penser que l'heterogeneite des globulines myelo mateuses γA n'est pas exclusivement liee à un equilibre de polymerisation La tendance a la polymerisation pour rait varier d'un type moleculaire γA à l autre (Heremans) (20) et l'on peut supposer que chez un meme sujet le polymorphisme structural de l unite moleculaire monomere puisse entrai ner l apparition de plusieurs types de polymeres

Il faut enfin tenir compte du fait que l heterogeneite peut etre liee a la formation de complexes entre la glo buline myelomateuse et l albumine (Heremans) (20) (Ballieux) (4) (Wal denstrom) (40)

References

1 Askonas B A Biochem J 1961 *79* 33

2 Askonas B A Fahey J L Nature (Lond) — 1961 *190* 980

3 Auscher C Guinand S Clin Chim Acta 1964 *9* 40

4 Ballieux R E Structurranalyse en clas sificatie von Béta 2 a paraproteinen Thèse Utrecht 1962

5 Bernier G M Putnam F W Biochem Biophys Acta 1964 *86* 295

Table 1 310 cases of myelomatosis arranged after age sex, electrophoretic pattern and after the type of cellular morphology

Age years		33—50		51—60		61—70		71—80		81—84		Type of cellular morphology
Sex		male	female	male	female	male	female	male	female	male	female	
Electro phoretic pattern	α increase	—	—	2	2	—	1	—	—	—	—	I Mature
	β increase	—	1	4	6	5	9	5	7	1	—	
	γ increase	8	6	23	12	21	29	17	13	6	3	
	No changes	1	1	1	2	—	—	2	1	—	—	
Electro phoretic pattern	α increase	1	—	—	1	2	—	1	1	—	—	II Immature
	β increase	6	2	11	9	21	13	7	10	2	2	
	γ increase	2	1	5	1	8	6	2	3	1	—	
	No changes	—	—	1	—	2	—	—	—	—	—	
Total per cent		9		26		38		22		5		

form immature plasma cells dominate the picture and the bone marrow reticulum is strongly hyperplastic Syncytial formations are numerous In the both main types typical "myeloma cells are found characterized of great often single nucleoli Inclusion bodies (Russel bodies) azur rods and the variable blueish red colouring of the protoplasm are typical findings

In the electrophoretic pattern pathological values have been accepted only if they reach outside of the normal variations (total amount)

Other methods of examination do not demand comment or detailed description

The material is collected in Table 1—1

I *The material arranged after age sex electrophoretic pattern and after the type of the cellular morphology (Table 1)*

The myelomatosis is a typical disease of age and 64 per cent of the cases are to be found at the age of 51—70 years of age The mean age was 64 years by the first examination Males are more often affected than females (54 per cent) The youngest man is 41 years of age and the youngest woman was 33 years of age The oldest case was a man 84 years of age The distribution of age and sex does not seem to be correlated neither to the cellular morphology of the bone marrow nor to the changes of the electrophoretic pattern

The *middle life span of 156 cases (information of the missing 154 cases has not been obtained)* runs up to 3 1/2 years after the diagnosis was made There is no difference between the sexes *The changes of the electrophoretic pattern* have no influence on the life span On the contrary the life span of immature myelomatosis is considerably shorter than that of mature myelomatosis and 72 per cent of the immature cases resulted in death within 2 years

From the Outpatient Department of Sodersjukhuset
Stockholm Sweden

Myelomatosis

A clinical review of 310 cases

BY NILS G NORDENSON

During the years 1932—1963, a material from more than 650 cases of myelomatosis has been collected The exact diagnosis has been made by sternal puncture and all cases have shown a specific and pathognomonic picture in the puncture specimen On the basis of this exact morphological diagnosis the patients have been carefully examined in all respects An invaluable help has been given me by many medical clinics in Sweden 310 cases have been examined and they give a basis for a systematic elaboration This material seems to be one of the greatest ones, which has been collected on one hand

Principles of work The division of the material has been made A) after the degree of the maturity of the myelomatosis — viz mature or immature plasma cell picture in the bone marrow and B) after the electrophoretic pattern — viz cases with increase of resp alpha beta and gamma globulin in the blood serum and cases without disturbance in this pattern

In the clinical picture the following different symptoms have been recorded I) the distribution of sex and age more over the life span, II) the proteinemia and the occurence of protein in the urine — non specific or specific (Bence Jones' type), III) the different types of changes in the skeleton, IV) the changes in the peripheral blood and V) the alterations of the sedimentation velocity and the disturbance of the function of the kidneys estimated by the increase of the non protein nitrogen

These five different types of clinical symptoms have been correlated to the changes of the bone marrow morphology and the electrophoretic pattern In general the symptoms of myeloma tosis are well known and in this respect the material gives no new aspects But correlated to another in the manner above mentioned interesting features can be elucidated

The mature myelomatosis is characterized of mature plasma cells and the engagement of the other reticular cells is less pronounced Syncytia are seldom to be found In the immature

beta type (5 g per cent) The highest value is found in a mature myelomatosis of the gamma type (14.5 g per cent)

In 87 per cent protein occurs in any form in the urine In 65 per cent albumine and in 22 per cent Bence Jones protein was found

In mature myelomatosis albuminuria is a common finding (72 per cent) Protein of the Bence Jones type is more frequent among immature myelomatosis There is no difference in the frequence of this protein in myelomatosis of the beta and of the gamma type also in alpha myelomatosis and cases that have no changes

Table II C The frequence of normal and abnormal protein in the urine in 310 cases of myelomatosis arranged after the electrophoretic pattern and the type of the cellular morphology

		Protein pos in urine	Bence Jones protein pos in urine	Type of cellular morphology
Electrophoretic pattern	α increase	5	2	I Mature
	β increase	24	9	
	γ increase	82	25	
	No changes	6	2	
Electrophoretic pattern	α increase	6	2	II Immature
	β increase	57	20	
	γ increase	20	6	
	No changes	2	1	

Table II D 310 cases of myelomatosis with normal and abnormal protein in the urine occuring in the various types of the disease (electrophoretic pattern and cellular morphology) Figures in parentheses = cases without protein in the urine

Electrophoretic pattern	3.4—6.9	7.0—7.9	8.0—9.9	10—14,5	Type of cellular morphology
			Protein pos in the urine		
α increase	1	1	2	1	I Mature
β increase	5 (2)	4 (2)	8 (10)	7	
γ increase	4 (5)	7 (6)	49 (33)	22 (12)	
No changes	2 (1)	3	1	— (1)	
α increase	2	1	2	1	II Immature
β increase	6 (1)	2 (1)	34 (21)	13 (5)	
γ increase	2 (1)	2 (1)	11 (10)	2	
No changes	2	1	—	—	
			Bence Jones protein pos in the urine		
α increase	1	—	1	—	I Mature
β increase	1	—	3	5	
γ increase	3	2	11	9	
No changes	—	2	—	—	
α increase	1	—	1	—	II Immature
β increase	3	2	13	2	
γ increase	1	2	2	1	
No changes	1	—	—	—	

II *Changes of the amount of blood related to the cellular maturity and Bence-Jones' protein in the urine correlated to the cellular maturity and the electrophoretic pattern (Tables II —II D)*

Mature myelomatosis are most common and occur in 60 per cent of the cases Myelomatosis of the gamma-type is found in 52 per cent, those of beta- and alpha-type occur in resp 40 and 4, and 4 per cent of the cases have no changes in the electrophoretic pattern 74 per cent of mature myelomatosis belong to the gamma type and 69 per cent of the immature to the beta type Myelomatosis of the alpha type is equally represented in the mature and the immature form Myelomatosis without electrophoretic changes are more frequent in mature forms (73 per cent)

Increased blood protein (more than 8 g per cent) is found in 79 and hypoproteinemia (less than 7 g per cent) in 11 per cent Immature myelomatosis have more often increased blood protein than mature forms (67 per cent) Myelomatosis of the gamma type have hyperproteinemia in 45 per cent but only 32 per cent of the beta type have increased blood protein Decreased or normal amount of blood protein is more common in mature myelomatosis Myelomatosis without changes of the electrophoretic pattern always has normal or a decreased amount of blood protein The lowest value of the blood protein is however found in a case of immature myelomatosis of the

Table II A 310 cases of myelomatosis arranged after electrophoretic pattern and after cellular morphology

			Type of cellular morphology
Electrophoretic pattern	α increase	5	I Mature
	β increase	38	
	γ increase	138	
	No changes	8	
Electrophoretic pattern	α increase	6	II Immature
	β increase	83	
	γ increase	29	
	No changes	3	

Table II B 310 cases of myelomatosis arranged after the amount of total blood protein, after the electrophoretic pattern and after the cellular morphology

Total blood proteine in g per cent		5—7	7 1—7 9	8—9 9	10—14 5	Type of cellular morphology
Electrophoretic pattern	α increase	1 (6 4)	1	2	1	I Mature
	β increase	7 (min 5 6)	6	18	7	
	γ increase	9 (min 5 4)	13	82	34 (max 14 5)	
	No changes	3 (min 5 9)	3	1	1	
Electrophoretic pattern	α increase	2 (min 5 6)	1	2	1	II Immature
	β increase	7 (min 5 0)	3	55	18	
	γ increase	3 (min 5 7)	3	21	2	
	No changes	2 (min 6 2)	1	—	—	
Total		34 (11 %)	31 (10 %)	185 (59 %)	64 (20 %)	

Table IV 310 cases of myelomatosis arranged after various peripheral blood changes electrophoretic pattern and after the cellular morphology

		Anemia	Thrombo cytopenia	Granulo cytopenia	Plasma cells	Leukemoid picture	Coincidence with "blood diseases": Lymphatic leukemia	Myelo blastic leukemia	Pernicious anemia	Type of cellular morphology
Electro phoretic pattern	α increase	3	2	3	1	1	—	—	—	I Mature
	β increase	23	21	16	3	2	1	—	—	
	γ increase	78	80	47	8	8	1	1	1	
	No changes	4	3	3	—	1	—	—	—	
Electro phoretic pattern	α increase	5	3	3	—	1	—	—	—	II Immature
	β increase	51	55	31	2	5	2	1	2	
	γ increase	20	21	11	1	2	1	—	—	
	No changes	2	—	—	—	—	—	—	—	

combined bone changes are more frequent in myelomatosis of the gamma type Cases lacking any skeleton changes are more common in myelomatosis of the gamma type The same concerns the alpha type of the disease and cases without alterations in the electrophoretic pattern

IV *Various changes of the peripheral blood correlated to the different types of myelomatosis (the maturity of the cellular morphology and the changes of the electrophoretic pattern) Table IV*

Changes of the blood in myelomatosis are usually combined Anemia and diminished platelet count occur in 60 per cent Granulocytopenia is found in 37 per cent In only 5 per cent of the cases plasma cells are found Leukemoid blood picture occurs in 7 per cent

Immature myelomatosis show more changes in the peripheral blood than mature forms Findings of plasma cells and leukemoid blood pictures are more frequent in the mature form of the disease Mature myelomatosis of the gamma type shows a high frequence of blood changes as well as immature myelomatosis of the beta type In cases of mature myelomatosis without changes in the electrophoretic pattern alterations in the peripheral blood are common

The coincidence of "blood diseases" and myelomatosis is unusual and this combination is specially seen in immature myelomatosis of the beta type

V *Changes of the sedimentation velocity and the non protein nitrogen arranged after the cellular maturity and the type of the electrophoretic pattern Table V*

Low values of the sedimentation velocity (below 30 mm in one hour) are

Table III 310 cases of myelomatosis arranged according to different bone changes occuring in the various types of the disease (electrophoretic pattern and cellular morphology)

Art of bone changes		Osteo porosis only	Gun shots only	Osteo porosis and Gun shots	Mixed changes	No skeletal changes	Type of cellular morphology
Electro phoretic pattern	α increase	—	1	2	2	—	I Mature
	β increase	9	5	13	7	4	
	γ increase	8	40	34	39	17	
	No changes	—	2	2	4	—	
Electro phoretic pattern	α increase	2	—	3	1	—	II Immature
	β increase	23	3	29	26	2	
	γ increase	1	8	10	10	—	
	No changes	—	—	2	1	—	
Total		43	59	99	90	23	

in the electrophoretic pattern Proteinuria of any form is more common in gamma myelomatosis of the mature type but more frequent in beta-myelomatosis of the immature form

There is no difference in the frequence of absence of proteinuria correlated to myelomatosis of various types of the electrophoretic pattern and the degree of the maturity as well as the amount of the total blood protein

Albuminuria is more common with increased total blood protein but is the opposite with decreased blood protein, the Bence Jones protein being more common

Albuminuria occurs in 91 per cent of myelomatosis of the beta- and the gamma types All cases of the alpha type of the disease have albuminuria In cases of myelomatosis missing changes in the electrophoretic pattern albuminuria is found in 73 per cent

III *Various bone changes (seen in X ray) in myelomatosis arranged according to the different types of the disease (the maturity of the cellular morphology and the changes of the electrophoretic pattern) Table III*

In 94 per cent of the whole material changes in the skeleton were found

Combined changes were most common (63 per cent) pure osteoporosis was found in 14 per cent and "gun shots" only in 17 per cent Pure osteoporosis is frequent in immature myelomatosis Mature types of the disease more often show "gun shots" only Combined changes are more frequent in mature myelomatosis Lack of any bone changes is more common in the mature form

Myelomatosis of the gamma type has more frequent skeleton changes than the beta type Pure osteoporosis is more common in myelomatosis of the beta type "Gun shots" as well as

ritz) Perhaps further studies of the morphology of the plasma cell will discover other pathological forms New details of the electrophoretic pattern will fully elucidate this interesting question

Myelomatosis is a systemic disease (reticulosis) specially in its immature form which occur in 40 per cent of all cases There is a widespread affection of the primitive reticular cell of the whole body The development of a local plasmocytoma in a myelomatosis or a plasma cell leukemia make this conception true There can hardly be a metastatic process The myeloma cell has all signs of malignity

The myelomatosis is a disease of age and there are no cases younger than 33 years of age The middle life span is about 3 1/2 years but it is definitely shorter in immature myelomatosis (up to 2 years)

Mature myelomatosis is most common as well as the gamma type of the disease Among the mature forms the gamma type dominates but the beta type is more usual in the immature myelomatosis

Hyperproteinemia is frequent in mature myelomatosis and in its gamma type Hyperproteinemia is more common in immature forms of the disease

Albuminuria occurs principally in cases with hyperproteinemia but the Bence Jones protein is more often found in cases with low blood protein

Skeleton changes are very frequent (91 per cent) The changes are usually combined Cases lacking bone changes are seen mainly in the gamma type of the disease Pure osteoporosis is frequent in the immature forms but "gun shots only are more often found in mature myelomatosis

Changes in the peripheral blood are usual and they are generally combined In immature forms of the disease they are most common Anemia and diminished platelet count are found in 60 per cent Findings of plasma cells are rare (5 per cent) and they occur specially in mature myelomatosis Only one case of plasmacell leukemia has been observed but a fully clinical examination was not obtained Leukemoid peripheral blood pictures occur mainly in mature myelomatosis The coincidence of "blood diseases" and myelomatosis is rare and is seen in immature forms of the beta type

The sedimentation velocity is always high and only 3 per cent of the cases showed a rate below 30 mm in one hour

Severe damage of the function of the kidneys was found in only 4 per cent

Myelomatosis is an interesting and fascinating disease This material shows its manifold symptomatology With all symptoms present the diagnosis is easy but essential symptoms can be lacking Several symptoms can be correlated to the maintypes of the myelomatosis Sometimes is this correlation confusing and unexpectable For instance in immature myelomatosis *a priori* more severe clinical picture ought to be expected In cases with

Table V 310 cases of myelomatosis arranged after sedimentation velocity, non protein nitrogen, electrophoretic pattern and after the type of the cellular morphology

		Sedimentation velocity (mm/l hour)				Non proteine nitrogen (mg per cent)				Type of cellular morphology
		—30	31—50	51—100	100—175	—40	41—50	51—100	100—300	
Electro phoretic pattern	α increase	—	—	2	3	2	2	—	1	I Mature
	β increase	—	—	10	28	19	14	5	—	
	γ increase	3	4	43	88 (173)	69	33	31	5 (225)	
	No changes	1	—	4	3	3	1	2	2	
Electro phoretic pattern	α increase	—	—	4	2	1	—	3	2	II Immature
	β increase	4	6	20	53	32	28	21	2	
	γ increase	1 (16)	—	2	26 (175)	15	9	3	2 (300)	
	No changes	1	—	1	—	1	—	2	—	

rare and found in 3 per cent High values (above 100 mm in one hour) occur in 80 per cent

In general, there is no correlation between the alterations of the sedimentation velocity and the maturity of the myelomatosis or the changes of the electrophoretic pattern However low values are found in immature forms of the disease The alpha-type has always high values as well as the beta type of the immature form

Changes in the function of the kidneys estimated after the increase of the non-protein nitrogen (NPN) show that a normal function (NPN below 40 mg per cent) is found in 50 per cent Severe damage in the function of the kidneys (NPN above 100 mg per cent) occurs in only 4 per cent In general there is no definite correlation between NPN and the degree of the cellular maturity or the changes of the electrophoretic pattern

Comments and summary

Myelomatosis viz multiple myeloma is a specific disease affecting the plasmacellular system It is characterized of a variegated clinical picture with five different main symptoms pathological plasma cells formation alterations in the blood proteins incl formation of Bence Jones protein skeleton changes changes of the peripheral blood and damage of the function of the kidneys In this material all these five symptoms occur in 30 per cent

In regard to the principles or work the material does not give any information of cases without positive changes in the sternal puncture but with other clinical symptoms of a myelomatosis

There seems to be a relationship between myelomatosis makro globulinemia and "paraproteinemia" (Waldenström) But the distinction of these three entities has been critizised (Und

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Alkeran® (Melphalan) in the Treatment of Myelomatosis

By Aa Drivsholm M D and Aa Videbæk M D

A large number of drugs have been tried in the treatment of myelomatosis but with meagre results (cf review by Drivsholm 1965 (6)) L phenylalanine nitrogen mustard (Alkeran® Melphalan) is among the most recent and more promising drugs This agent was synthesized by Bergel and Stock (1) in 1953 and in its racemic form (sarcolysin) it was applied to the treatment of myelomatosis for the first time by Blokhin Larionov Perevodchikova Chebotareva and Merkulova (4) in 1958

This is a report on 4 years experience of Alkeran in the treatment of myelomatosis A preliminary report on part of the material has previously been published by Videbæk (17)

Material and Method

Material

The study comprises all symptomatic cases admitted to the two above mentioned medical departments during the period 1 2 1961—31 1 1965 a total of 5 patients From the final analysis the following patients were excluded 1) 10 patiens followed for less than 3 month 2) 8 patients who could not be followed regularly in the named departments 3) 1 patient who had cancer of the lung besides myelomatosis and 4) 1 patient who had been treated also with cyclophosphamide during the observation period The remaining 55 patients were followed by one of us throughout the treatment period By the end of the observation period 19 of these patients had died

The diagnostic criteria were 1) Myeloma cells in a bone marrow specimen and 2) either an M component in the serum (immunoelectrophoresis) or typical skeletal lesions (X ray)

The distribution of the material by sex age (at the time of diagnosis) and type of M component is given in Table 1

Principle of Treatment

Alkeran was administered *either as serial treatment* or as *permanent treatment* In the former used during the early part of the study period the dosage was 10 mg daily for a maximum of 6 days and intervals of 3 weeks were allowed to elapse between each

divergences in the electrophoretic pattern from the usual ones viz alpha-myelomatosis and cases with normal electrophoresis, the clinical picture ought to be more penetrated

References

Sturgis, C C Hematology Springfield Illinois U S A 1955
Undritz E personal communications
Undritz E Schw Med Wchnschr 1957 87 1431
Waldenstrom J Acta Med scand 1944 117 216
Waldenstrom J Schw Med Wchnschr 1948 78 927

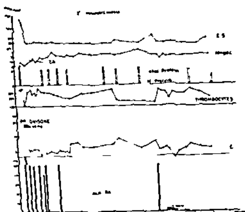


Fig 1 Biochemical changes during treatment with *Alkeran® for 44 months in a case of* γA myelomatosis initially with hypercalcaemia (CA)

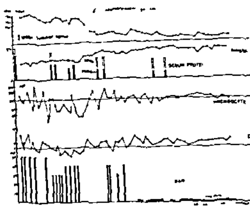


Fig 2 Biochemical changes during treatment with Alkeran® for 48 months in a case of γG myelomatosis

Table 1 *Material*

55 patients: 26 ♂, 29 ♀

Average age 62 0 ± 9.3 years (37—81 years)

\+ M component in the serum 53 pts: γG 39 pts; γA 13 pts; Bence Jones 1 pt

— M component in the serum 2 pts: Hypogammaglobulinaemia; Immunoparalysis

Table 2 *Treatment with Alkeran (55 pts)*

Duration Average 21 4 ± 13 1 months (3—48 months)
Total dose Average 802 ± 561 mg (110—2350 mg)
Daily dose Average 1.32 ± 0 59 *mg (0 1—2 4 mg)*

Table 3 *Therapeutic effect*

	+effect	—effect
A Alkeran	13	3
B Alkeran + local radiotherapy	9	2
C Alkeran + prednisone	14	1
D Alkeran + prednisone + local radiotherapy	11	2
	47	8

course The permanent treatment was started on 5 mg daily for 10 days after which the maximum dose was 2 5 mg daily The maintenance dose was in all cases adapted according to the platelet count, white cell count and haemoglobin concentration During the early part of the treatment, these parameters were checked once weekly, later every 3 or 5 weeks

The average total and daily doses administered to the 55 patients are listed in Table 2 which also gives the duration of treatment

The treatment was instituted in all cases with Alkeran Patients in severe pain were also given short courses of radiotherapy to the isolated, painful skeletal foci If there was vital indication for prednisone therapy (hypercalcaemia with cerebral confusion or severe nephropathy with rapidly deteriorating renal function and imminent uraemia), the treatment was supplemented by prednisone

In 42 of the 55 patients the Alkeran therapy was instituted within 6 weeks after myelomatosis had been diagnosed Six patients had been treated with urethane prior to the Alkeran therapy but not immediately before The remaining patients had not previously been treated with cytostatics

Evaluation of Therapeutic Effect

The criteria of a therapeutic effect were

1) > 20 % decrease in the *M protein* concentration in the serum
2) > 20 % increase in the *albumin* concentration in the serum (in the event of a decreased albumin concentration)
3) > 20 % decrease in the ESR
4) > 20 % increase in the haemoglobin concentration (without transfusion)
5) > 20 % decrease in an elevated *serum creatinine*
6) Decreasing *proteinuria*
7) Normalization of an elevated *serum calcium*

The patients' statements concerning decreased *pain* and a possibility of *increased mobility* after the institution of Alkeran therapy were recorded but never used as the only criterion of a therapeutic effect

Results

The *therapeutic effect* is given in Table 3 in which the series is grouped by method of treatment A therapeutic effect is recorded, if only one of the above mentioned 7 criteria was fulfilled In the majority of these cases however several of the criteria were fulfilled Out of the 8 patients in whom no effect could be traced 7 had an M component in the serum of the γG type, while the eight had only immunoparalysis Of the 47 patients who gave a primary response to the treatment 10 were again showing signs of increased disease activity at the end of the observation period Two of these patients had not been treated as intensively as might have been desired because they did not cooperate properly Out of the remaining 8 4 had been treated with Alkeran and prednisone 4 with Alkeran only Fig 1 gives the

Table 5. *Side effects of Alkeran therapy (55 pts.)*

Expected	Trombocytopenia (< 100 000/µl)	33 pts.
	Leukocytopenia (< 2 000/µl)	34 pts.
	Increased anaemia	16 pts.
Unexpected	Hair loss	1 pt.
	Exanthema	3 pts.
	Bone pain	3 pts.
	Nausea, vomiting, diarrhoea	6 pts.

Discussion

As emphasized int. al. by Waldenström (19) it is difficult to evaluate the therapeutic effect in myelomatosis as the course and prognosis in the untreated patient are extremely varying. Moreover, the varying course as well as the poor prognosis (18) usually necessitate individual treatment by several drugs.

The criteria used in the present study to assess the therapeutic effect correspond rather accurately to those used by Waldenström (19) in his analysis. We did not include the patients' statements concerning less pain and fatigue, increased mobility and ability to work etc. in our assessment of the therapeutic effect — even though such changes were striking — because it is so difficult to compare subjective sensations and to rule out a suggestive influence upon the patients. In many instances the analgesic effect of Alkeran was very pronounced and manifest as early as a week or two after the medication was started. Statistical calculations of the survival curve representing the treated patients were not carried out in the present study because of the fairly short follow-up period and the lack of a comparable untreated series of patients with myelomatosis. A fall in the percentage of plasma cells in bone marrow specimens was not used as a criterion of a therapeutic effect owing to variations from one preparation to another from the same specimen and in various specimens removed within the same 24 hours (7).

The assessment of the effect of a drug is particularly difficult when other drugs are being administered at the same time. In certain cases the demand of the best possible treatment owing to the poor prognosis of the diseases made it necessary to supplement Alkeran therapy with prednisone and local radiotherapy.

However, neither of these two treatments appears to entail a general improvement in the prognosis of myelomatosis (5).

The distribution of the present series by sex, age and type of M component corresponds accurately to the distribution in a larger Danish series of myelomatosis from 1964 (5).

The favourable effect of Alkeran in myelomatosis corresponds roughly on the whole to that found by others (2, 3, 4, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 20). As none of the 8 patients with

Table 4 *Therapeutic effect obtained*

Alteration in per cent	20—39 %	40—59 %	60—79 %	> 80 %	Total
Decrease in M protein	5	15	14	4	38 (53)
Increased in albumin	3	2	2	6	13 (31)
Fall of E S R	8	11	13	12	44 (54)
Increase in Hb	4	4	0	1	9 (35)
> 20 % decrease in elevated serum creatinine					14 (17)
Declining proteinuria					9 (37)
Normalization of serum calcium					4 (5)

course of the disease in one of the patients who primarily went into complete remission, clinically as well as biochemically After 21 1/2 months without any form of treatment, the patient developed a recurrence and succombed despite repeated Alkeran medication This patient had initially exhibited a prompt decrease in an elevated serum calcium on prednisone Alkeran treatment Such an effect was observed on Alkeran alone in one out of 5 cases with hypercalcaemia The therapeutic effect of Alkeran is often of slow onset This is illustrated in Fig 2 which also shows a marked increase in haemoglobin on Alkeran medication

In Table 4 an attempt is made to grade the therapeutic effect in the total series The figures in brackets signify the number of the patients who showed alterations of the various parameters The M component decreased by more than 40 % in 3/4 of the patients, and the albumin concentration often increased The haemoglobin concentration usually rose but nevertheless showed a declining tendency in 16 cases In 5 cases the decrease in serum creatinine was obtained on Alkeran alone, while the remaining 9 patients were treated with Alkeran plus prednisone In 1 patient the serum creatine decreased from 7 5 to 2 5 mg/100 ml on Alkeran alone In 2 of the 9 patients with decreasing proteinuria, this decrease occurred on Alkeran alone, while the other 7 patients were receiving Alkeran as well as prednisone. There was no correlation between the average daily dose of Alkeran and changes in the parameters E S R, M protein, and albumin concentration

Healing of osteolytic skeletal lesions did not occur in any case on Alkeran.

The *side effects* of Alkeran are shown in Table 5 The thrombocytopenia and leukocytopenia were often pronounced and yielded but slowly and often only partially after discontinuation of Alkeran The tendency to leukocytopenia and thrombocytopenia was pronounced in patients with impaired renal function Two patients died of severe infection perhaps caused by an Alkeran induced leukocytopenia Fatal haemorrhages due to severe thrombocytopenia were not observed As already mentioned the haemoglobin concentration showed a declining tendency in 16 patients during the treatment All the unexpected side effects were mild

the degree of proteinuria an effect was obtained in 85 % of the patients (Table 4). Serum creatinine as well as serum calcium and the proteinuria could be appreciably reduced by Alkeran alone. In 10 cases there were signs of increased disease activity by the end of the observation period.

Side effects in the form of thrombocytopenia and leukocytopenia were often ascertain and were especially pronounced in patients with impaired renal function. Other side effects were rare and mild.

It is concluded that Alkeran is applicable with success in the treatment of myelomatosis. However it should be used only in the treatment of myelomatosis which has been definitely diagnosed and showing manifest signs of disease activity. The present study did not answer the question whether an initiated Alkeran medication therapy should be continued after a therapeutic effect has been obtained.

Acknowledgement

We are greatly indebted to Jorgen Clausen M.D. Head of the Institute of Neurochemistry Copenhagen for the immunoelectrophoretic studies.

Alkeran® was generously supplied by Burroughs Wellcome & Co.

References

1 Bergel F & Stock J A. A.R. Brit Emp Cancer Campgn 31 6 1953
2 Bergsagel D E., Sprague C C. Austin C & Griffith K M. Cancer Chemother Rep *21* 87 1962
3 Bernard J Seligmann M & Danon F Nouv Rev franc. Hémat. 2 611 1962
4 Blokhin N Larionov L F Perevodchikova N I Chebotareva L & Merkulova N Ann NY Acad Sci *68* *1128* 1958
5 Drivsholm Aa Acta med Scand *176* 509 1964
6 Drivsholm Aa Myelomatose Thesis p 81 Munksgaard Copenhagen 1965
7 Drivsholm Aa & Clausen J Acta med Scand *175* 609 1964
8 Eridani S Taglioretti D & Tiso R Gital Chemioter *11* 93 1964
9 Hoogstraten B Abstracts Xth Congr int Soc Haematol Stockholm *D 32* 1964
10 Johansson B Abstracts Xth Congr int. Soc Haematol Stockholm D 31 1964
11 Moeschlin S & Jubin E Praxis 53 1471 1964
12 Norin T Unio internat Contra Cancrum Acta 20 382 1964
13 Osserman E F Proc Amer Ass Cancer Res 2 50 1963
14 Rordam P Proc. Xth Congr Europ Soc. Haematol Strasbourg 1965 (In press)
15 Speed D E Galton D A G & Swan A Brit med J I 1664 1964
16 Swan A Proc Eight Congr Europ Soc Haematol. Vienna 1961 Part I p 162 Karger Basle
17 Videbæk Aa Proc IX Congr int Soc Haematol Mexico City 1962 *vol I* 593 1964
18 Videbæk Aa & Johansen H Dan med Bull 3 174 1956
19 Waldenstrom J Brit med. J *I* 859 1964
20 Waldenstrom J Proc. Xth Congr Europ Soc Hematol Strasbourg 1965 (In press)

lacking effect of Alkeran had type γA myelomatosis, patients having myelomatosis of this type are perhaps on the whole more responsive to Alkeran therapy than patients with M protein of the γG type in the serum This possibility was emphasized already by Moeschlin & Jubin (11) Apparently some patients are resistant to the treatment from the very outset However, Alkeran should be continued for a long time, before a therapeutic effect can be excluded (cf Fig 2) Other patients appear to develop resistance to the treatment for some unknown reason, so that they have to be switched over to another cytostatic As already mentioned it may be risky to discontinue Alkeran, although the patient has entered clinical and biochemical remission (cf Fig 1) This has also been emphasized by Waldenstrom (19)

The favourable effect of Alkeran upon an impaired renal function is beyond doubt Patients with impaired renal function appear to be more apt than others to develop leukocytopenia and thrombocytopenia on Alkeran therapy, but if the medication is combined with prednisone Alkeran is better tolerated Therefore it is recommended to supplement the treatment with prednisone in the presence of definitely impaired renal function Prednisone also appears to be indicated as an adjuvant in patients with severe proteinuria and in patients with severe hypercalcaemia

The side effects of Alkeran are as already mentioned, few and harmless if overdosage is avoided Therefore cases of myelomatosis with pancytopenia, due exclusively to the basic disease, should also be treated with Alkeran, in small doses, the anaemia being treated, in severe cases, by transfusion of packed erythrocytes (and possibly prednisone), the leukocytopenia and thrombocytopenia with prednisone However, the side effects may be fatal, so that accidentally diagnosed cases of myelomatosis should probably not be treated with Alkeran, until signs of disease activity appear Patiens with an M protein component in the serum, in whom the diagnosis is not definite should not be treated until the diagnosis of myelomatosis has been confirmed If these precautions are observed, Alkeran is an exellent, relatively non toxic cytostatic for the treatment of myelomatosis

Summary

Fifty five patients with myelomatosis were followed for 3—48 months during treatment with Alkeran® The average follow up period was 21 4 ± 13 1 (S D) months The total dose ranged from 110 to 2350 mg the average daily dose being 1 32 ± 0 59 (S D) mg Patients with severe bone pain received initially radiotherapy of isolated skeletal foci Patients having alarming hypercalcaemia with cerebral confusion and patients with severe nephropathy were treated concurrently with prednisone Nineteen of the patients had died by the end of the observation period

Assessed on the basis of the alterations in the serum concentration of M protein and albumin the L S R Hb serum creatinine serum calcium and

incubated for 3 10 and 20 minutes after which 0 2 ml fibrinogen solution or 0 2 ml normal citrated plasma was added

The presence of fibrinolytic split products were demonstrated immunochemically (12 13)

The myeloma globulins were classified immunochemically by Bachmann and Laurell (2) and purified by Hansson et al (6)

Results (Table I II and III)

Of the 63 patients examined 39 had myeloma globulin type γG (γG myeloma) 14 type γA (γA myeloma) 6 type lightchain protein (γμ myeloma) 3 had 2 abnormal globulins (γA+γG γG+γμ γA+γμ myeloma) and one patient type γD globulin (γD myeloma) recognizable in serum on paper electrophoresis (9) Seventeen patients had bleeding symptoms (nose bleeding bleeding from oral mucosa menorrhagia purpura and subcutaneous haematoma) at the time of blood sampling or just before and of these 9 had γG myeloma 6 γA 1 γμ and one patient γA+γμ myeloma

The mean values and ranges for some coagulation factors in different types of myeloma are given in table I

Coagulation defects were about equally common in patients with and without bleeding symptoms (Table II)

The distribution of normal low and high levels of platelets prothrombin AHF and pathologic fibrinolytic activity did not vary with the type of myeloma (Table III) The fibrinogen values (Table I and III) were high 0 37—1 21 g per cent) in 28 of the 39 patients with γG myeloma Of these 28 patients the antithrombin titre was increased in 16 as well as in 3 patients

Table I Mean values and ranges of some coagulation factors in different types of multiple myelomas

Type of myeloma globulin	Number of patients examined	Coagulation time (8—14 min)	Bleeding time (Duke) (1—5 min)	Prothrombin +f VII+f X (f IISP)	Factor V (80—190 %)	AHF (f VIII) (60—160 %)	B factor (f IX) (60—160 %)	Plasminogen activity (60—140 %)	Fibrinolytic activity lysed area in mm² (0—50) plasma	(0—70) euglob precip	Fibrinogen g/100 ml (0 23—0 31)	Number of patients with prolonged thrombin time
γG	39	15 (7—28)	4 (1—30)	97 (21—140)	110 (40—200)	158 (24—500)	118 (48—243)	112 (61—190)	55 (0—203)	107 (0—139)	0 48 (0 23—1 21)	19
γA	14	13 (5—21)	3 5 (2— 6)	81 (50—108)	111 (00—193)	116 (30—210)	88 (50—130)	105 (51—173)	16 (0—115)	50 (0—193)	0 31 (0 10—0 62)	0
γμ	6	13 (8—16)	3 (2— 6)	116 (68—172)	141 (79—236)	220 (78—324)	144 (118—200)	108 (91—121)	18 (0—40)	91 (51—100)	0 55 (0 28—1 15)	0
γD	1	11	8	116	120	195	—	91	0	61	0 37	0
γA+μ	1	10	4	58	15	87	—	88	10	10	0 81	1
γA+γG	1	10	2	91	120	163	83	127	0	67	0 32	0
γG+μ	1	21	1	160	106	83	75	170	0	80	0 42	0

From the Coagulation Laboratory (Head Inga Marie Nilsson, MD) the Department of Clinical Chemistry (Head Carl Bertil Laurell MD) and the Department of Medicine (Head Jan Waldenström MD) General Hospital, Malmö University of Lund Sweden.

Coagulation studies in different types of myeloma [1]

By Jan-Erik Nilléhn and Inga Marie Nilsson

The occasional bleeding in patients with multiple myeloma is often due to thrombocytopenia (7) But in such patients the clot retraction may be retarded, the one stage prothrombin time prolonged and the antithrombin titre increased (5, 8, 11, 22), i e all abnormalities which may contribute to impaired haemostasis Some authors (5, 11, 22, 23) ascribe these coagulation defects to the myeloma globulins

The purpose of present investigation was to ascertain whether any association exists between the coagulopathies particularly the antithrombin effect and different myeloma globulins

Material and methods

Clinical material 63 patients 24 men and 39 women aged 38—86 of the department of medicine Malmo general hospital were examined The diagnosis of myeloma was based on roentgenological skeletal changes demonstration of pathological proteins (M components) in the plasma and the presence of an increased number of atypical plasma cells in the bone marrow

Methods The blood was collected with silicone technique and citrated plasma and serum were prepared as described previously (15 19) As a rule the coagulation studies included determination of the coagulation time in glass and plastic tubes bleeding time recalcification time of citrated plasma prothrombin consumption prothrombin and factor VII factor V one stage prothrombin time AHF (f VIII) and haemophilia B factor (f IX) fibrinogen assays of fibrinolytic activity plasminogen activity and inhibitors of plasminogen activation were performed by methods described elsewhere (16 17 18, 19 The thrombin time was determined in the way described earlier (12) and was said to be prolonged when it exceeded the control with 10 seconds in citrated plasma samples coagulated with thrombin (Topostasin Roche 3 NIH ml) The isolated globulins were dissolved in a phosphate buffer (pH 7 0 0 05 M) in 0 5 M NaCl solution and tested for their antithrombin effect in the following system a) 0 2 ml 0 4 g per cent human fibrinogen solution (Kabi) + 0 2 ml of globulin solution (or buffer for control) + 0 2 ml of thrombin solution (3 NIH per ml) b) a mixture of 0 2 ml globulin solution (or buffer for control) and 0 2 ml of thrombin solution (3 NIH ml) was

[1] This investigation was supported by grants from the Swedish Medical Research Council and the Medical Faculty University of Lund

The AHF values were increased in 24, normal in 35 and low in 3 patients (Table I and II).

Discussion

It is clear from the results given above that a variety of defects may occur in the coagulation mechanism of patients with multiple myeloma. Of our 63 patients 17 had bleeding symptoms. The coagulation defects did not appear to vary in frequency or type with the presence or absence of bleeding symptoms (Table II). As expected thrombocytopenia was common. The fibrinolytic activity was often abnormally increased and the fibrinogen level was elevated in half of the patients (Table I and III). Of special interest was the high antithrombin titre in multiple myeloma (5, 11, 22, 23). It was found in 20 patients including 19 with γG myeloma and one patient with γA+γμ myeloma. In none of the patients could a low fibrinogen value explain the prolonged thrombin time. The fibrinogen value was normal in 3 of the patients and high in 17 with a high antithrombin titre. The fibrinogen level could not explain the high antithrombin titre (10) because it was normal in the other patients with myeloma and high fibrinogen level. We also determined the antithrombin titre in myocardial infarction, pneumonia and other infectious diseases with high fibrinogen values (0.45—0.95 g per cent) but none of them had a prolonged thrombin time. No correlation seems therefore to exist between high antithrombin titre and high fibrinogen levels in myeloma patients. Individual differences in the thrombin binding capacity of the fibrinogen molecule may exist but this was not investigated.

The absence of a demonstrable anticoagulant effect in the first stage of coagulation excluded the possibility of a circulating anticoagulant of type antithrombin II (heparin+heparin co-factor) (4).

A possibility hitherto not considered in myeloma patients is that the amount of split products arising on digestion of fibrinogen by plasmin (1) might be sufficient to inhibit the conversion of fibrinogen to fibrin. In 12 of the patients with a high antithrombin titre an abnormally high fibrinolytic activity in plasma was found and comparative immunoelectrophoresis revealed fibrinolytic split products in 4 of 8 studied. This suggests that split products may help to explain the high antithrombin titre in some myeloma patients but they cannot be responsible for the prolonged thrombin time in most of the patients.

Like other workers in this field (5, 11, 22) we thought that the abnormal globulin occurring in myeloma was responsible for the prolonged thrombin time. Isolated γG globulin (6) from 3 of our patients with prolonged thrombin time was tested in different systems with γG globulin concentration between 1.4 and 1.8 g per cent. These γG globulins were found to have no antithrombin effect. This was incompatible with the findings reported by earlier investigators (5, 11, 22, 23) who however did not work

Table II Frequency of some coagulation defects in 17 patients with and 46 without bleeding symptoms

	Prolonged coag time	Prolonged bleed time	Decreased number of platelets	Decreased prothrombin	Prolonged one stage prothrombin time	Decreased AHF	Decreased fibrinogen	Increased fibrin lysis
With bleeding symptoms	8	4	9	6	4	3	3	[illegible]
Number of patients studied	16	17	17	17	17	17	17	17
Without bleeding symptoms	15	6	18	8	7	0	0	25
Number of patients studied	45	40	38	44	14	43	44	44

Table III Distribution of normal, low and high values of some coagulation factors in patients with γG, γA and γμ myeloma

Type of myeloma globulin		Platelets	Prothrombin	Fibrinogen	Fibrinolytic activity	Split products	Antithrombin titre	AHF
γG	Normal	17	27	10	17	8	19	23
	Low	13	7	—	—	—	—	1
	High	3	3	28	21	7	19	12
γA	Normal	7	8	9	8	1	14	9
	Low	4	6	7	—	—	—	2
	High	1	0	1	[illegible]	0	0	3
γμ	Normal	3	3	3	2	0	6	1
	Low	2	1	0	—	—	—	—
	High	1	2	3	4	1	0	[illegible]

with *γG myeloma* with normal fibrinogen value (0 23—0 34 g per cent) The patient with γA+γμ myeloma also had a high antithrombin titre and a high fibrinogen value (0 81 g per cent) In the patients with γA myeloma the fibrinogen values ranged from 0 19 g per cent to 0 62 per cent but in none of the 14 patients with γA or the 6 with γμ myeloma was the antithrombin titre increased Nineteen patients were studied for fibrinolytic split products In 8 of them the sera contained such products Sternal punctate from 5 patients without any demonstrable split products in the serum from venous blood showed pronounced fibrinolytic activity in sternal plasma on unheated fibrin plates (150—300 mm²) Fibrinolytic split products were also found in sternal serum without (all 5 patients) and with ε ACA (3 patients)

The inhibitors of plasminogen activation by urokinase or streptokinase in 54 patients studied proved normal in 44 decreased in 3 and increased in 7

14 Nilého J E and Nilsson I M To be published 1966
15 Nilsson I M Blomback M and v Francken I Acta Med Scand 159 35 1957
16 Nilsson I M Blomback M and Ramgren O Acta Med Scand 170 665 1961
17 Nilsson I M and Olow B Acta Chir Scand 123 247 1962
18 Nilsson I M and Olow B Thromb Diathes haemorrh 8 297 1962
19 Paraskevas M., Nilsson I M. and Martinsson, G Scand J clin Lab Invest 14 138 1962
20 Perry S J Lab clin Med 61 411 1963
21 Sirridge M S Bourman, K S and Garber P E Arch intern Med 101 630 1958
22 Uehlinger E Helv med Acta 16 508 1949
23 Viala J J Thouverez J P., Belleville J., Revol L. and Croizot, P Hémostase III 303 1963

with purified preparations, but was in accord with the findings of Perry (20) in his investigation of plasma fractions from myeloma patients

The thrombin binding capacity of the γG globulin may, of course, be destroyed during preparation of the pure globulin Besides our test system may not be sensitive enough to detect the defects Further investigation of the myeloma globulins is therefore desirable

The antithrombin effect noted in multiple myeloma is still obscure but it may be due to a) fibrinogen degradation products b) different thrombin binding capacity of fibrinogen or c) the presence of some other protein in increased amounts in particularly γG myeloma

Sirridge et al (21) described 2 myeloma patients with fibrinolysis In our myeloma material the frequency of fibrinolysis was remarkably high compared with fibrinolysis in a clinical material of coronary infarction and pneumonia with high and normal fibrinogen values (14) This difference is difficult to explain It may be due to the involvment of the bone marrow in multiple myeloma The presence of split products in sternal serum and/or in venous blood may depend of the degree of the activity of the myeloma as may the high fibrinogen and AHF (f VIII) (3)

Summary

In 63 patients with multiple myeloma 17 were found to have bleeding symptoms Coagulation defects of the same type occurred in patients with and without bleeding symptoms Fibrinolysis was found in half of the patients and high fibrinogen values were equally common

The thrombin time was prolonged in 20 of the patients investigated In 19 of them the disease was classified immunochemically as γG myeloma and in one as γA+γμ myeloma The high antithrombin titre could not be ascribed to the myeloma globulin or to the high fibrinogen values noted

References

1 Alkjaersig N Fletcher A P and Sherry S J clin Invest *41* 917 1962

2 Bachmann R and Laurell C B Scand J clin Lab Invest 15 Suppl 69 p 11 1963

3 Egeberg O Scand J clin Lab Invest *14* 230 1962

4 Favre Gilly J Creyssel R Thouverez J P Revol L and Croizat P Hemostase *III* 325 1963

5 Frick P G Amer J clin Path 25 1262 1955

6 Hansson U B Laurell C B and Bachmann R Acta Med Scand Suppl 445 1966

7 James Th N Ann intern Med *39* 1281 1953

8 Klesper R Achenbach W and Lich R Deutsch Arch klin Med *206* 59 1959

9 Laurell C B Laurell S and Skoog N Clin Chem 2 99 1956

10 Loeliger A and Hers J Thromb Diathes haemorrh *1* 499 1957

11 Luscher E and Lobhard A Schweiz med Wschr *79* 598 1949

12 Nilehn J E and Nilsson I M Scand J Haemat *1* 313 1964

13 Nilehn J L and Robertson B Scand J Haemat 2 267 1965

14 Nilehn J L and Nilsson I M To be published 1966

15 Nilsson I M Blomback M and v Francken I Acta Med Scand *159* 35 1957

16 Nilsson I M Blomback M and Ramgren O Acta Med Scand *170* 665 1961

17 Nilsson I M and Olow B Acta Chir Scand *123* 247 1962

18 Nilsson I M and Olow B Thromb Diathes haemorrh *8* 297 1962

19 Paraskevas M., Nilsson, I M and Martinsson G Scand J clin Lab Invest *14* 138 1962

20 Perry S J Lab clin Med *61* 411 1963

21 Sirridge M S Bourman K S and Garber P E Arch intern Med *101* 630 1958

22 Lehfinger F Helv med Acta *16* 508 1949

23 Viala J J., Thouverez, J P., Belleville J., Revol L. and Croizot P Hemostase *III* 303 1963

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Cryoglobulinuria. Studies of a cryo-Bence Jones protein [1]

By CHESTER A. ALPER, M.D.

Over three decades ago Wintrobe and Buell (21) described the first cryoglobulin, a serum protein which precipitated in the cold. Since this observation was made, numerous examples have been reported, the majority occurring in the serum of patients with multiple myeloma or Waldenström's macroglobulinemia. In most of these cases the cryoprecipitate or cryogel has been shown to consist of a γG or γM paraprotein or a complex of γM paraprotein with normal γG (5). Thus cryoglobulinemia is a form of what Waldenström has termed gammopathy (19), usually of the monoclonal variety (20).

Whereas the vast majority of reported cryoglobulins have been 150,000 or more in molecular weight with sedimentation constants of 7S or greater, one cryoprotein of 3.6S recovered from the urine of a patient with multiple myeloma has been described (18). The relationship of this protein to the immunoglobulin system (6) was not defined, nor did the patient's urine give a positive Bence Jones heat test.

In the present study a similar urinary cryoprotein was isolated and investigated, particularly with regard to its place among the immunoglobulins.

Materials and methods

Case report. The patient was a 59 year old white female whose chief complaint on admission was severe back pain. On physical examination she had a blood pressure of 180/95 and muscle spasm and tenderness over the lower thoracic spine but no other abnormalities. X-ray studies revealed osteolytic lesions of the skull, ribs and several thoracic vertebrae as well as generalized undermineralization of the bones. Abnormal laboratory findings included 11.0 gm% hemoglobin, an erythrocyte sedimentation rate (corrected) of 27 (Wintrobe method) and a blood urea nitrogen of 30 falling to 14 mg%. The white blood cell count and differential were normal as was the platelet count. Paper electrophoresis of her serum proteins revealed a total protein of 5.3 gm% with albumin 4.1, α_1 globulin 0.2, α_2 globulin 0.2, β globulin 0.5 and γ globulin 0.3 gm%. Thus in addition to hemodilution her pattern showed possible *in vivo* hemolysis and hypogammaglobuline

[1] The immunoglobulin nomenclature used here is that recommended in the WHO Bull. 1964, *30*, 447.

m a Exam nat on of smears of tl e bone mar row asp rate re ealed about a % plasma cells of wh ch many were mmature Ur nary prote n excret on was 2 gm per 24 hours total volume was about 9 0 ml) and on concentrat on and paper electrophores s th s was seen to cons st *almost ent rely of an M* component of rap d gamma mob l ty Dur ng *concentrat on* of tl e ur ne n the col l a cryog l form ed and th s f nd ng pro pted the *stud es n tl s report* The Bence Jones heat te t of tl e pat e ur ne was pos e

Tl e pat ent s course was compl cated by tl orac c sp nal cord compress on requ r ng lam nectomy and an ep sode of acute left ventr cular fa lure wh ch responded pron ptly to 1 p tal s and mercur al d uret cs Her ane m a was cons derably mpro ed on parenteral androgen tl erapy About one year follow ng l er n t al d agnos s of m ult ple myeloma she d ed uddenly No autopsy was performed

I u f cat on of the ur n ry M component Tl e u nary M component was pur f ed by l al s aga nst cold runn ng tap water pre c p a on w th 60 % saturated ammon um ulfate preparat e electrophore s on l ev kon !! at r om ten peratu e us ng barb tal buffer p H 8 6 I 2 =0 1 and f nally DEAE cellulose ch omat graph (I u n g an n al wash w tl tr s p l o pl ate b ffer 0 005 M pH 8 0 f ll we l b grad ent elut on w tl tl s l uffer *an l 0 3 M o oba c sod u n p l o p l ate* at n te nperature Tl e bulk of tl e prote n w eluted w tl tl e start ng buffer l ut th re a al o a n oderate ad l t onal peak af er g a l nt lut on was begun Pooled mater al f n tl e f rst peak was u ed for further u ly

l h gel ele t opho es s Th s procedure wa carr e l out u ng Sn tl es o g nal l or tal n tl nl to All fra t ons of ur nary p ote n were subjected to tl s anal s s and fo co parat e purpo es pu f d ormal γG *a p r f ed γ A* M-compone t and a pur f ed γM pro fron a pa nt w l Walden trom s acr lol ul en a were elec rop l resed at l a e t e Tl e polypep l de cl a struc u e wa n e t ated in l r zontal tarch gel *ectr l e s as lescr bed by E l l an and* l ul k l n d u g *buffers* and reagents u l l S tl es et al 1C

Effects of prote n concentrat on molar ty pH and reduc ng agent on cryogel nd cryo-prec p t te format on To test the cr t cal prote n concentrat on for the cryoprote n phenon enon solut ons were prepared of the pur f ed ur nary prote n n solon c sal ne and d t lled water These solut ons ranged from 1 0 to 5 0 gm% in 0 5 gm% prote n steps To e aluate the effect of solute molar ty pH and red c ng agent 0 2—0 5 ml port ons of tl e pur f ed prote n at 5 7 gm % were d alyzed aga nst 250 ml of ar ous buffers as outl ned n Tables I and II

Imm unochem l stud es Analyses of the pur f ed ur nar prote n were carr ed out by tl e Ouchterlony tecl que (12) w th l gl ly pec f c ant sera to γG γA γM kappa chains and lambda cha ns Ant gens were tested at w dely d fferent concentrat ons n eacl sys tem Tl e ur nary prote n was also anal zed n mmunoelectrop l ores s (14) and n starch gel n munoelectrophores s (13) us ng the same ant sera

Carbohydrate and p ote n determ n t ons Carbol ydrate was determ ned by tl e anthrone method (10) and prote n oncentrat on l y the Fol n C ocalteu n ethod (8 w th fructo e and pur f ed normal γG as standards

Ultracentr fugal analys s Samples of tl e pur f ed prote n at e eral concentrat *ons n* 0 15 M p H 0 p l osphate buffered sal *ne were analyzed n a* Sp nco Model E ultracentr fuge Tl e observed sed mentat on constant was corrected for ten pe ature solute concentra t on and prote *n concentrat on* The tempera *ture of eacl run was* tl e ne ghborl ood of 20 C

Results

The starch gel electrophoretic pattern of the purified urinary protein is shown in Figure 1 Normal γG and two M components one γA the other γM were subjected to the same ana lysis for comparison It is seen that the urinary protein consists of a major rapid b nd and three fainter even more rap d bands In starch gel immuno

From The Blood Grouping Laboratory of Boston, The Richard C. Curtis Hematology Research Laboratory, Peter Bent Brigham Hospital, and the Departments of Medicine, Harvard Medical School and the Children's Hospital Medical Center, Boston, Massachusetts, U.S.A.

Cryoglobulinuria. Studies of a cryo-Bence Jones protein[1]

By CHESTER A. ALPER, M.D.

Over three decades ago Wintrobe and Buell (21) described the first cryoglobulin, a serum protein which precipitated in the cold. Since this observation was made numerous examples have been reported, the majority occurring in the serum of patients with multiple myeloma or Waldenstrom's macroglobulinemia. In most of these cases, the cryoprecipitate or cryogel has been shown to consist of a γG or γM paraprotein or a complex of γM paraprotein with normal γG (5). Thus cryoglobulinemia is a form of what Waldenstrom has termed gammopathy (19) usually of the monoclonal variety (20).

Whereas the vast majority of reported cryoglobulins have been 150 000 or more in molecular weight with sedimentation constants of 7S or greater, one cryoprotein of 3.6S recovered from the urine of a patient with multiple myeloma has been described (18). The relationship of this protein to the immunoglobulin system (6) was not defined, nor did the patient's urine give a positive Bence Jones heat test.

In the present study, a similar urinary cryoprotein was isolated and investigated, particularly with regard to its place among the immunoglobulins.

[1] The immunoglobulin nomenclature used here is that recommended in the WHO Bull 1964 *30* 447.

Materials and methods

Case report. The patient was a 59 year old white female whose chief complaint on admission was severe back pain. On physical examination, she had a blood pressure of 180/95 and muscle spasm and tenderness over the lower thoracic spine but no other abnormalities. X-ray studies revealed osteolytic lesions of the skull, ribs, and several thoracic vertebrae as well as generalized undermineralization of the bones. Abnormal laboratory findings included 11.0 gm% hemoglobin, an erythrocyte sedimentation rate (corrected) of 27 (Wintrobe method) and a blood urea nitrogen of 30 falling to 14 mg%. The white blood cell count and differential were normal as was the platelet count. Paper electrophoresis of her serum proteins revealed a total protein of 5.3 gm% with albumin 4.1, α_1 globulin 0.2, α_2 globulin 0.2, β globulin 0.5 and γ globulin 0.3 gm%. Thus, in addition to hemodilution her pattern showed possible *in vivo* hemolysis and hypogammaglobuline-

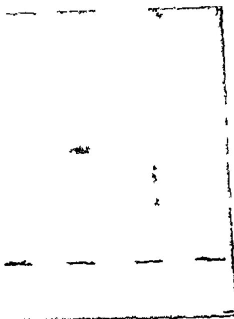


Figure 1 Starch gel electrophoretic patterns of normal γG, the urinary cryoprotein, a purified γA M component and a purified γM protein from a patient with Waldenstrom's macroglobulinemia from left to right. The electrophoresis was performed at pH 8.2. The γM protein characteristically fails to enter the gel.

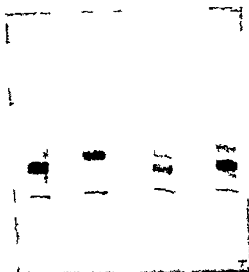


Figure 3 Polypeptide chain patterns of normal γG, the urinary cryoprotein, a γA M component, and a γM macroglobulinemia protein. The pattern of the urinary cryoprotein shows material with the mobility of light polypeptide chains only. Electrophoresis was performed in starch gel with formate buffer pH 3.0 containing 8M urea and 2 mercaptoethanol.





Antigenic analysis of the urinary cryoprotein. The left hand center well (A) contains an [illegible]serum to kappa light polypeptide chains and the right hand center well (A) contains an [illegible]serum to lambda type chains. Peripheral wells 1 and 4 on both sides contain the [illegible] wells 2 and 5 contain a reference lambda type protein and wells 3 and 6 a reference kappa type protein. There is no reaction of the urinary protein with kappa antiserum but a strong precipitin band forms with the anti lambda antiserum which spurs with the reference lambda protein.

Table I *The influence of pH and electrolyte concentration on the formation of cryogel or cryoprecipitate* [1]

Phosphate Buffer Concentration	pH 5.0	pH 6.0	pH 7.0	pH 8.0	pH 9.0	pH 10.0
0.15 M		± precipitate	++ precipitate	+++ precipitate	gel	
0.10 M			semi gel	++ precipitate	semi gel	
0.05 M			gel	gel	gel	
0.01 M		gel	gel	gel	gel	semi gel
0.005 M	gel	gel	gel	gel	gel	gel

[1] All samples were at a protein concentration of 5.7 gm % and were kept at 4°C for 16 hours. The absence of an entry indicates no change in solution.

Table II *The influence of 2-mercaptoethanol (2 ME) on the formation of cryogel or cryoprecipitate*

	0.1 M 2 ME	0.05 M 2 ME	0.005 M 2 ME
0.1 M Phosphate Buffer	+ precipitate	+ precipitate	+ precipitate
0.005 M Phosphate Buffer	gel	gel	gel

All solutions at a protein concentration of 5.7 gm % and pH 8.0

electrophoresis using an anti lambda chain antiserum, each of these bands gave a precipitin arc which fused with that of its neighbor in a reaction of identity. In this technique there was no material which reacted with antisera to γG, γA, γM or kappa chains nor was there any reaction with an antiserum against whole human serum. Figure 2 depicts the reactions of the urinary protein with an antiserum specific for kappa chains (left hand portion) and another specific for lambda chains (right hand portion). For comparison reference kappa and lambda type Bence Jones proteins are included. It is clear that the urinary protein is of type lambda. The failure of this protein to react in Ouchterlony analysis or immunoelectrophoresis with antisera specific for γG, γA or γM suggests that it consists only of light polypeptide chains.

That this was the case was supported by the polypeptide chain pattern in urea mercaptoethanol starch gel electrophoresis as shown in Figure 3. Here it is seen that whereas normal γG and the two purified serum M components consist of both light and heavy polypeptide chains, the urinary cryoprotein is made up entirely of material with the mobility of light chains.

Ultracentrifugal analysis revealed that the urinary protein had a corrected sedimentation constant of 3.4S consistent with that of light chain dimer. Although the material gave a single peak in this analysis there was some asymmetry towards the meniscus suggesting the presence of some material of lower S value.

Summary

Studies of a cryoprotein isolated from the urine of a patient with multiple myeloma showed that it consisted only of lambda light polypeptide chains and was a typical Bence Jones protein. A solution of the protein formed a gel, a precipitate or remained unchanged at 4°C depending upon protein concentration and the electrolyte molarity and pH of the solution.

Acknowledgements

The author wishes to thank Misses Marianna Marshall and Domenica Paci and Mrs. Rosanne Stein for skilled technical assistance. He is also grateful to Dr. John Harter and Mr. John McNiff for performing the ultracentrifugal analysis, to Dr. Ezio Merler for providing certain antisera and to Dr. Edward Franklin for providing the reference Bence Jones proteins. *This work was partially supported by grants GM 09107, AM 00965-11 and AI 0 8 [illegible] from The United States Public Health Service.*

References

1 Burtin P, Hartmann L, Fauvert R and Grabar P: Rev Franç Études Clin et Biol *1* 1 1956

Clamp J R, Bernier G M and Putnam F W: Biochim Biophys Acta *86* 149 1964

3 Edelman G M and Gally J A: J exp Med *116* 207 1962

4 Edelman G M and Poulik M D: J exp Med *113* 861 1961

5 Franklin E C: Progr Allergy *8* 58 1964

6 Heremans J F: Les globulines sériques du système gamma. Leur nature et leur pathologie. Masson (Paris) 1960

7 Korngold L and Lipari R: Cancer *9*, 183 1956

8 Lowry O H, Rosebrough N J, Farr A L and Randall R J: J biol Chem *193* 265 1951

9 Migita S and Putnam F W: J exp Med *117* 81 1963

10 Morris D L: Science *107* 254 1948

11 Müller-Eberhard, H J and Kunkel H G: *J exp Med 104* 253 1956

12 Ouchterlony Ö: Acta path et microbiol scand *32* 231 1953

13 Poulik M D: J Immunol *82* 502 1959

14 Scheidegger J J: Int Arch Allergy *7* 103 1955

15 Smithies O: Biochem J *61* 629 1955

16 Smithies O, Connell G E and Dixon G H: Amer J Hum Gen *14* 14 1962

17 Sober H A and Peterson E A: Fed Proc *17* 1116 1958

18 Varriale P, Ginsberg D M, and Sass M D: Ann Int Med *57* 819 1962

19 Waldenström J: Harvey Lect *56* 211 1961

20 Waldenström J: Progr Allergy *6* 320 1962

21 Wintrobe M M and Buell M V: Bull Johns Hopkins Hosp *52* 156 1933

The carbohydrate content of the protein was 0.9 %.

When the purified urinary protein in 0.15M saline was kept at 4°C for 16 or more hours, only those solutions at a protein concentration of 3.5 gm % or higher formed gels.

The effects of pH, solute molarity and reducing agent on the formation of cryogel or cryoprecipitate are shown in Tables I and II. Thus, this protein would either gel or precipitate in the cold and which, if either occurred, was influenced by several factors. Gel formation occurred maximally at pH 9 and was favored by low ionic strength. Precipitate formation appeared to be promoted by high ionic strength and a pH of 7—8. It appeared that 2-mercaptoethanol had no effect on cryogel or precipitate formation.

Discussion

All previously studied cryoglobulins have been shown to consist of complete immunoglobulin molecules or complexes of complete molecules (5). Classical Bence Jones proteins are known to consist of only a portion of the complete molecule, the light polypeptide chains (3). The present studies indicate that it is possible for a protein consisting only of these light chains to be a cryoglobulin. They also suggest that molecular size is not an essential factor in cryoprecipitability.

In all respects the protein reported here was a typical Bence Jones protein at least in terms of the characteristics studied (1, 3, 7, 9). The absence of visible non-lambda chain contaminants on starch gel and starch gel immuno-electrophoresis strongly suggest that cryogel and cryoprecipitate formation were properties of the protein itself and not of some contaminant. This is further supported by the low carbohydrate content (2) of the purified protein.

The phenomena of cryoprecipitation and cryogel formation are not well understood. The work of Franklin and co-workers (5) suggests that weak non-covalent bonds are formed in the cold leading to aggregation or gel formation. The present studies support this concept in that the gelling of the cryo-Bence Jones protein was promoted by low molarity of electrolyte and high concentration of protein. The fact that neither maximal gelling nor precipitation occurred around the isoelectric point of the protein (perhaps pH 6—7 to judge from its mobility on paper electrophoresis at pH 8.6) suggests that the phenomena require particular amino acids in a specific steric arrangement to be ionized or not ionized and are not reflections of simple insolubility. Since the same protein behaved as either cryoprecipitate or cryogel, the close relationship of these two forms of cryoprotein is suggested.

In view of the conditions necessary for gelling or precipitation to occur it seems highly unlikely that the patient could have been affected in any way by the curious properties of her urinary M component. However, had the urine concentration of her Bence Jones protein been higher and had the conditions for gelling or precipitation been somewhat different, the clinical consequences might have been catastrophic.

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References

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Fig 1 Case 1 Flaming plasmacells

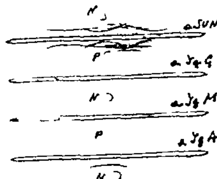


Fig 3 Case 1 Immunoelectrophoresis
N=normal serum
P—serum of the patient



Fig 2 Case 1 Flaming plasmacell in electron microscope

was hypogammaglobulinemia with *almost total absence of IgA* and absence of IgM This shows that the *flaming cells plasmocytom is not always* accompanied by IgA hyperdysprotemenia One might think that the IgA protein remains in the cytoplasms without passing into the circulation but the IEph of bone marrow homogenate treated with ultrasounds (where presumably *the* cells have been broken) has not exibited any *difference from the blood serum*

The cytochemistry shows that the flaming zones are not pyroninophile and are almost completely PAS negative No BJ exists in the urines

From this it can be deduced that 1) the correlation between flaming plasmacells and IgA dysproteinemia is not obligatory 2) The flaming plasmacells of this case seem to contain degenerate matter which has lost many of the characteristics of the hemoproteins

Case 2 — Fa Lina female 56 years old kept in the University Medical Clinic of Firenze from 13 to 23 6-1963

Clinical course

Subject obese for 2 years lumbar sacral pains Slow development sensitive to therapy with prednisone (Lack of news of present state)

Skeleton X ray

Fractures and multiples zones of osteolysis

Sternal biopsy

a) May Grumwald Giemsa marrow rich with total invasion of plasmacells with wide

Clinica medica generale dell Università di Firenze
Direttore prof E. Greppi

Unusual Morphologic and Humoral Conditions in the field of Plasmocytoms and M-dysproteinemia

By RENATO DI GUGLIELMO

The increasingly extended and detailed study of plasmocytomatous diseases and of the cases of so called M dysproteinemia continually offers the possibility of detecting unusual new conditions, which often contribute to improving our knowledges not only of these diseases but also of certain problems of general physio pathology

The classical optic microscopic cytomorphology still retains its full importance in indicating such exceptional conditions, which will later be studied more fully with the modern techniques of the electron microscopy and of immunophysicochemistry

From our collection of more than 200 observations we shall refer briefly to 5 cases which seem to us particularly unusual

Case 1 — Am Renato male 65 years old kept in the Hospital of Empoli from 1 7 to 18 8 1964

Clinical course

For 2 months pains of the ribs and lower part of the spine Slow clinical course fairly sensitive to therapy with prednisone+urethane+anabolic drugs (The patient is still living)

Skeleton X ray

Multiple zones of osteolysis

Sternal biopsy

a) May Grunwald Giemsa marrow rich in cells 95 % plasmacells nearly all "flaming" (fig 1)

b) Unna Pappenheim the flaming zones of the cytoplasms are not pyroninophile

c) PAS lightly diffused positivity of the cytoplasms the flaming zones are less coloured or discoloured

d) Electron microscopy corresponding to the flaming zones the ergastoplasm appears dilated in enormous sacs containing amorphous matter (fig 2)

Proteins study

a) Serum total proteins g 5.3/100 cc Paper electrophoresis A 62 α_1 7.1 α_2 9.3 β 12.5 γ 9.3 % Immunoelectrophoresis (IEph) IgG slightly diminished IgA traces IgM absent (fig 3) IEph of bone marrow homogenate treated with ultrasounds (with the purpose of breaking the cells) identical with that of the serum

b) Urines negative the research of the Bence Jones proteins IEph traces of IgG

Comments

In a case of typical bone plasmocytom with flaming plasmacells the total serum proteins were low normal there



Fig 6 Case 3



Fig 8 Case 3 Danielli s staining

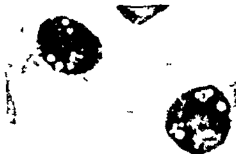


Fig 7 Case 3

Skeleton X ray

Diffuse decalcification collapse of 2 lum bar vertebrae

Sternal biopsy

a May Grunwald Giemsa marrow rich in cells with 50 % plasmacells of these 61 % contained characteristic round enclosures, usually multiple *inside* the nuclei (fig 6) surrounded by a border of dark material (fig) 1 5 % showed such enclosures *also* in the cytoplasms 1 % *only* in the cytoplasms 36 5 % was without enclosures

b PAS and Sudan black B the enclosures did not colour

c) Danielli (for the proteic substances) the enclosures were clearly positive (fig 8)

Proteins study

a) Serum total proteins g 7 8/100 cc Paper electrophoresis A 61 4 α_1 6 2 α_2 11 0 β 8 5 γ 12 9 % IEph quantitative diminution of the 3 Ig fractions

b) Urines albuminuria no *B J* IEph slight traces of IgG notable albuminuria

Comments

In a case of bone plasmocytom with out paraproteinemia with diminution of the 3 Ig fractions and without B J proteinuria 61 % of the marrow plas macells contained endonucleic enclo sures of proteic nature The phenome non reached a degree and a diffusion never before noted it is not easy to say whether the incapacity of the plas macells to empty the proteins into the circulation can facilitate the appea rance of proteic drops in the interior of the nuclei

Case 4 — Ma Cesare male 66 years old kept in the University medical Clinic of Fi renze from 7 3 to 11-4 1962

Clinical course

In 1954 fracture on the right femur conse quently bone pains treated with cobalt the rapy but without effect Intense wasting tu mours in the right ribs Treated in the Clinic with large doses of prednisone (mg 150 daily ×4 days 100×3 75×2 50×20) urethane



Fig 4 Case 2



Fig 5 Case 2

cytoplasms often confluent containing an enormous number of small spheres which sometimes are also on the nuclei There are numerous giant polyploid cells with a monstrous nucleus these also contain innumerable small spheres in the cytoplasm (fig 4 and 5)

b) Sudan black B and PAS the spheres are negative

c) Danielli (for the proteic substances) the spheres are strongly positive

Proteins study

a) Serum total proteins g 6 0/100 cc Paper electrophoresis A 54 2 α_1 5 2 α_2 1 1 β 13 9 γ 15 7 % IEph completely normal

b) Urines no Bence Jones proteins

Comments

In a case of typical bone plasmocytom without demonstrable alterations of serum proteins and without BJ proteinuria there are numerous monstrous polyploid cells in the cytoplasm of these as in that of the other plasmacells there is a great number of droplets which show cytochemically a proteic nature

The analogy of this observation with that presented by Brucher and Meiser at the Congress of the Swiss Hematology Society in 1965 is evident the sole differences are represented by the proteic enclosures, present in our case but not in the Swiss one, and by the B J proteinuria absent in our case and strongly represented in the Swiss case

The finding of the giant plasmacells with monstrous nuclei is very rare we consider that the cases of this type deserve to be pointed out in order to see whether there are other characteristics apart the morphological ones which make it possible to classify them in a special variety of plasmocytoms

Case 3 — Po Emilio male 62 years old out patient of the Medical Clinic from November 1964 and then admitted to the Urological Clinic of Firenze in April 1965

Clinical course

For 15 months albuminuria without apparent causes without hyperazotemia For 3 months violent lumbar pains which disappeared with anabolic drugs and an orthopedic corset Consequently a serious increase of the azotemia until the uremic coma the patient died in april 1965 after 21 months of illness A course of therapy with prednisone+urethane+anabolic drugs did not have any effect on the azotemia which steadily grew worse



Fig 11 Case 5

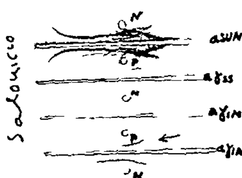


Fig 12 Case 5

γ 38.2%. IEph very important increase of IgA IgG and IgM normal (fig 12)

b) Urines no B J ILph albuminuria.

Comments

In a woman 46 years old a sure hypercellular and hypersplenomegalic leukemia was accompanied by a "major" IgA dysproteinemia It is an absolutely exceptional combination it is not easy to understand which clone of cells produces the paraprotein unless one thinks of the same leukemic cells which have a histioid appearance which partially reminds the cells of the reticulosarcoms

Summary

In this paper are briefly referred 5 observations which can be considered exceptional

Case 1 Bone plasmocytom very rich in flaming plasmacells *without* IgA paraproteinemia

Case 2 Bone plasmocytom with enormous cells which have monstrous nuclei and innumerable proteic droplets in the cytoplasms with *complete normality* of the serum proteins and no proteinuria

Case 3 Bone plasmocytom with plasmacells extraordinarily rich in *endonucleic* proteic enclosures *without paraproteinemia* and with diminution of the 3 Ig fractions

Case 4 Bone plasmocytom IgG with the presence in the cytoplasms of the plasmacells of innumerable *crystal like enclosures*

Case 5 Hypersplenomegalic *histio leukemia* with "major" IgA dysproteinemia

References

Brucher H., Meiser J Schweiz. med Wschr 95 1426 1965



Fig 9 Case 4



Fig 10 Case 4

(g 1 5 daily) anabolic drugs The results were extremely good on the general condition on the anemia on the proteins (see below) on the rib tumours (which completely disappeared) Further news is lacking

Sternal biopsy

Marrow rich in cells the normal tissue was well *preserved there were 18%* of myeloma cells Of these a good number showed in cytoplasms very many clear *rectangular* bodies which looked like crystals (fig 9) and which sometimes were found also on the nuclei (fig 10) (There was no cytochemical examination)

A second biopsy carried out after a month in greatly improved general and proteic conditions showed an almost equal number of plasmacells (15%) but the almost total disappearance of the enclosed bodies

Proteins study

a) Serum (8 3 1962) total proteins g 9 15 /100 cc Paper electrophoresis A 27 (2 4) α_1 8 3 (0 7) α_2 15 5 (1 1) β 11 (1 0) γ 38 2 (3 5) IEph IgG doubled in cathodic direction IgA and IgM normal

(4 4 1962, after mg 1900 of prednisone+ g 23 of urethane) total proteins g 5 7/100 cc Paper electrophoresis A 33 2 (1 89) α_1 7 8 (0 4), α_2 15 2 (0 8) β 11 4 (0 6) γ 32 4 (1 85) IEph unchanged

b) Urines no B J proteins

Comments

In a case of bone plasmocytoma with strong dysproteinemia *IgG many of* the marrow plasmacells showed very many crystal like enclosures Such enclosures which, as far as I know, have never been noted almost disappeared after a period of intense therapy with prednisone and urethane a therapy which, at the same time provoked a very important diminution of the paraprotein

Case 5 — Greek woman 46 years old admitted to the University Medical Clinic of Thessaloniki (Greece) in 1965

Clinical course

The patient suffered for some months from a *form of histioleukemia with hyperleukocytosis and enormous spleno hepatomegaly*

Skeleton X ray normal

Sternal biopsy

Total substitution of normal marrow with histioid granulous cells (fig 11) identical to the cells present in the peripheral blood There it was not possible to find plasmacells

Proteins study

a) Serum total proteins g 9 5/100 cc Paper electrophoresis A 35 α_1 5 1 α_2 8 5 β 13 4

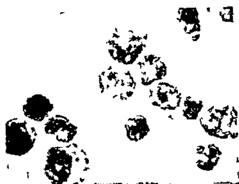


Fig 11 Case 5

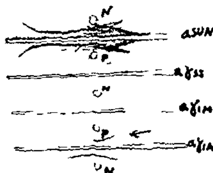


Fig 12 Case 5

γ 38 2 % IEph very important increase of IgA IgG and IgM normal (Fig 12)
b) Urines no B J IEph albuminuria

Comments

In a woman 46 years old a sure hypercellular and hypersplenomegalic leukemia was accompanied by a "major" IgA dysproteinemia It is an absolutely exceptional combination it is not easy to understand which clone of cells produces the paraprotein unless one thinks of the same leukemic cells which have a histioid appearance which partially reminds the cells of the reticulosarcoms

Summary

In this paper are briefly referred 5 observations which can be considered exceptional

Case 1 Bone plasmocytom very rich in flaming plasmacells *without* IgA paraproteinemia

Case 2 Bone plasmocytom with enormous cells which have monstrous nuclei and innumerable proteic droplets in the cytoplasms with *complete normality* of the serum proteins and no proteinuria

Case 3 Bone plasmocytom with plasmacells extraordinarily rich in *endonucleic* proteic enclosures *without paraproteinemia* and with *diminution of the 3 Ig fractions*

Case 4 Bone plasmocytom IgG with the presence, in the cytoplasms of the plasmacells of innumerable *crystal like enclosures*

Case 5 Hypersplenomegalic *histioleukemia* with "major" IgA dysproteinemia

References

Brucher H., Meiser J Schweiz med Wschr 95 1426 1965

From Medical Department B, the Department of Clinical Chemistry and the Department of Clinical Physiology, Bispebjerg Hospital, Copenhagen, Denmark

A Case of Myelomatosis with Normal Colloid Osmotic Pressure in Spite of Extremely High Serum Protein Concentration (Hyperviscosity syndrome due to aggregation of myeloma globulin molecules?)

By Mogens Bjorneboe and K. Birger Jensen

In many cases of myelomatosis the serum protein concentration is so high that it is hard to understand how the body is able to maintain a normal colloid osmotic pressure. The *calculated* pressure ranges far beyond normal levels.

By experimentally induced hyperimmunization in rabbits extremely high concentrations of γ-globulins can be produced (1). Examination of the plasma volume in these animals showed that the volume increases concurrently with the γ globulin concentration and consequently it is justifiable to presume that the body strives to maintain a normal colloid osmotic pressure by means of this mechanism (2, 3). Besides, direct measurement of the pressure in samples of serum from these animals, using Tybjærg Hansen's osmometer, reveals this pressure to be normal, in spite of great variations in the concentration of γ globulins (4). By studying more comprehensive series of patients with myelomatosis in whom measurements of the plasma volume and the concentration of myelomaprotein are made, it is possible to show that *no* correlation exists between these two values (5, 6).

Hence an increase in the plasma volume cannot be the only mechanism of control of the colloid osmotic pressure in this disease.

A study of the case reported below suggested to us that an aggregation of globulin molecules may contribute to the control of the colloid osmotic pressure in myelomatosis.

Supported by grants from the King Christian X Foundation and Vera and Carl Johan Michaelsen's Foundation.

Case report

A 51 year old man was admitted to this hospital on October 19th, 1964 and died here on November 6th, 1964. Apart from minor ill-

nesses he had been in good health until March 1962 when he was treated in his home with penicillin because of pneumonia In July 1964 and shortly afterwards he had two episodes of pneumonia and was again given penicillin A subsequent chest X ray showed infiltration in the right lung and he was admitted to the department of chest surgery of this hospital During his stay there he had severe epistaxis and when anaemia proteinuria and arterial hypertension were revealed he was transferred on the 26th of October to medical department B for further study He said that he had for some time been troubled by fatigue dyspnoea on exertion and dizziness Physical examination disclosed an enlarged liver extending 8 cm below the costal margin but no enlargement of the spleen and no palpable lymph nodes Blood pressure was slightly elevated Ophthalmoscopy on October 28th disclosed normal discs distended veins without segmentation numerous haemorrhages fanshaped or in streaks and some woollen" exudates The ophthalmologist described the findings as typical for Waldenstrom's macroglobulinaemia

On October 30th he complained of severe headache and pains in the finger joints and on November 4th of low back pain He became increasingly sleepy complained of impaired memory and reduced ability to concentrate He became progressively weak and dropped things he had in his hands

On November 5th he developed pneumonia with a slightly elevated temperature In spite of tetracycline therapy he died on November 6th Raynaud's phenomena or acrocyanosis were not observed during the hospital course

Clinical and laboratory investigations

Weight 8 kg Height 1"2 cm.

Blood pressures were 160/100 160/105 and *160 110 mm Hg*

Serum protein studies

Paper electrophoresis (on October 31st) total protein 18 6 g per 100 ml albumin 1 82 α_1 *0 23* α 0.36 β_1 0 33 β plus γ globulin 0 47 and an intermediary M component in the γ area of 15 40 g per 100 ml

Immuno electrophoresis[1] (on November 6th) revealed a γ-paraprotein with slow and intermediary mobility reduced IgA IgM and albumin slightly elevated α_2 macroglobulin A tendency to aggregation into bigger units was demonstrated The cold agglutinin titres were normal.

By ultra centrifugation analysis (on November 26th) of serum diluted to a total protein value of 1 4 g per cent 20 9 % of the protein showed 4 1 Svedberg units 78.5 per cent showed 6 1 and 0 6 % showed 16 6 Svedberg units[2]

Plasma volume determinations with ^{125}I labelled IgG globulin 5 020 ml, i e 84 4 ml per kg (normal value 40 2 ml per kg) (7)

Colloid osmotic pressure in plasma kindly determined by P Ingerslev M D the Department of Clinical Physiology (according to the method of Tybjarg Hansen) was 47 cm of water (normal range 34—46) The calculated pressure according to (4) was 61 cm of water

Lumbar puncture (on November 4th) pressure 240/110 mm of water The cerebro spinal fluid contained 2 white blood cells and 17 red blood cells per c.mm glucose 78 mg per 100 ml. Immuno electrophoresis of spinal fluid (on October 29th) was normal and revealed no signs of paraproteinoracchia

E S R varied between 145 and 154 mm/hour Haemoglobin 6 4—6 2 g/100 ml On October 27th red cells 2 18 mill/µl, haematocrit 20 per cent, MCV 92 mpl MCHC 32 g/100 ml platelets 24 000—10 000 per µl *White cells* 9 200 per µl Differential count 42 neutrophils 1 eosinophils 48 lymphocytes 1 monocytes and 5 per cent plasma cells The red blood cells showed pronounced agglutination which made the counting difficult This agglutination tendency disappeared by heating to 37° C

Alkaline phosphatases 3 8—3 6 *King Armstrong* units per 100 ml (normal range 3—10) Basal metabolic rate (on the 3rd and 4th of November) 115 and 119 per cent SGPT 1 4 *mU per hour per litre* (normal range <1.8)

[1] Kindly performed by J Clausen M D

[2] We are grateful to B Mansa cand pharm., who carried out this test

Thymol turbidity 0 71 (normal range <0 13) Serum bilirubin 0 6 mg per cent Prothrombin 62 per cent Serum calcium (on November 5th) 11 1 mg per 100 ml Serum phosphorus 4 5 mg per 100 ml Arterial blood (on October 31st) oxygen saturation 93 per cent pH 7 44 standard bicarbonate 23 5 m Eq/litre pCO_2 36 mm Hg Serum chloride 101 mEq/litre Serum sodium 130 mEq/litre Serum potassium 3 9 mEq/litre Serum haptoglobin 46 mg per 100 ml (normal range 18—190) Red blood cor puscle osmotic resistance was lowered Serum B_{12} 360 pg per ml Serum creatinine 1 8 mg per 100 ml Creatinine clearance 65 and 74 ml/min The Sia test was positive

Urine examination showed proteinuria 0 4 per cent Bence Jones protein no glycosuria Microscopic examination of urine revealed slight haematuria

Bone marrow aspirate (on October 28th) showed 35 per cent of plasma cells of abnormal structure with some binuclear types characteristic of myeloma

Blood culture (on November 6th) was negative as also culture from the spinal fluid

The electrocardiogram was normal

X ray films of the lungs taken on July 24th September 22nd and October 21st revealed an infiltration in the right upper lobe Films taken on November 5th showed numerous small infiltrations in both lungs

Intravenous pyelography was normal

Neurological examination on November 6th led to the suspicion of a paresis of throat and tongue E E G was abnormal with low frequent activity especially frontally

Post mortem examination revealed confluent bronchopneumonias in both lungs Microscopical examination showed intense plasma cell infiltration in the spleen the lungs and in particular in the bone marrow

Discussion

The present case of myelomatosis is characterized by an extremely high concentration of serum protein The colloid osmotic pressure, as calculated on the basis of the concentrations of albumin and γ globulin in serum ranges considerably beyond normal limits, whereas measurement of the actual pressure gives almost normal result (47 cm of water) This discrepancy may be explained by assuming that an aggregation of the myeloma globulins has taken place In this connection it is of interest to observe that in many respects the clinical picture in this case resembles the hyperviscosity syndrome which has recently been described by Smith et al (8), viz (1) bleeding, in particular from the mucous membranes of nose and mouth, and (2) disturbed vision, dilatation and segmentation of retinal veins, round haemorrhages papilloedema To these may be added (3) weakness, fatiguability, (4) peripheral oedema, decreased pulse pressure, (5) vertigo electroencephalographic changes syncope and convulsions and (6) distension of dependent veins and capillaries, dependent plethora

Our patient exhibits several symptoms which are similar to those observed in the above patients epistaxis, haematuria typical retinopathy the finding of aggregations of paraprotein by immuno electrophoretic studies pronounced rouleauxformation in the blood smears electroencephalographic changes and peculiar neurological findings

In this connection it should be mentioned that as early as in 1944 in his first description of Waldenstrom s macroglobulinaemia Waldenstrom ascribed these findings to hyperviscosity (9) Ott mentions in 1956 that the cal

culated colloid osmotic pressure differs markedly from the directly measured pressure in myelomatosis the same observation as we have made However, he does not ascribe this finding to aggregation of the protein molecules (11)

True enough we did not succeed in demonstrating this aggregation by ultracentrifugation but this may be due to the fact that the serum was extremely diluted prior to examination

Therefore we find it justifiable to assume that in our case of myelomatosis the colloid osmotic pressure is kept down by a combination of an increase in the plasma volume and an aggregation of myeloma globulin molecules

In addition it is only natural to explain the different mechanisms of control in the immunized animals and in the patient with myeloma by pointing to the fact that identical or almost identical molecules are polymerized much more easily than non identical molecules (10) Myeloma globulins are characterized by being homogenous whereas immuno globulins produced by immunization are composed by several different types of γ globulins

Summary

A case of myelomatosis with a concentration of para protein of 3.5 g per 100 ml is reported The colloid osmotic pressure measured is 47 cm of water whereas the calculated value is 61 The plasma volume is 64 4 ml/kg It is postulated that the patient s colloid osmotic pressure is maintained at normal values by means of a combination of increased plasma volume and aggregation of myeloma globulin molecules This hypothesis is substantiated by the fact that the clinical picture in this patient in certain respects resembles the "hyperviscosity syndrome" described by Smith et al

References

1 Bjorneboe M Serum proteins during immunization" Acta Path et Microbiol Scand., 20 221 (1943)
2 Bjorneboe M & M. Schwartz "Investigations concerning the changes in serum proteins during immunization" J Exp Med 110 259 (1959)
3 Bjørneboe M & S Jarnum "The changes in serum proteins and blood volume during immunization" J Exp Med. 113 1005 (1961)
4 Ingerslev P O A Larsen and N A Lassen personal communication
5 Gabuzda T G "The turnover and distribution of I131 labeled myeloma and macroglobulin proteins" J Lab Clin. Med., 59 65 (1962)
6 Andersen S B Metabolism of human gamma globulin (γ55 globulin)" Blackwell Scientific Publications Oxford 1964 pg 78
7 Ibid pg 36
8 Smith, E S Kochwa and L R Wassermann Aggregation of IgG globulin in vivo I The hyperviscosity syndrome in multiple myeloma Am J Med., 39 35 (1965)
9 Waldenstrom, J Incipient myelomatosis or essential hyperglobulinemia with fibrinogenopenia — a new syndrome?" Acta Med Scand 117 216 (1944)
10 Pauling L and H A Itano Molecular structure and biological specificity" 1957 pg 28
11 Ott, H Die Errechnung des Kolloidosmotischen Serumdruckes aus dem Eiweiss Spektrum und das mittlere Molekular gewicht der Serumeiweissfraktionen" Klin Wochenschrift 34 1079 (1956)

Thymol turbidity 0.71 (normal range <0.13). Serum bilirubin 0.6 mg per cent. Prothrombin 62 per cent. Serum calcium (on November 5th) 11.1 mg per 100 ml. Serum phosphorus 4.5 mg per 100 ml. Arterial blood (on October 31st): oxygen saturation 93 per cent, pH 7.44, standard bicarbonate 23.5 mEq/litre, pCO_2 36 mm Hg. Serum chloride 101 mEq/litre. Serum sodium 130 mEq/litre. Serum potassium 3.9 mEq/litre. Serum haptoglobin 46 mg per 100 ml (normal range 18—196). Red blood corpuscle osmotic resistance was lowered. Serum B_{12} 360 pg per ml. Serum creatinine 1.8 mg per 100 ml. Creatinine clearance 65 and 74 ml/min. The Sia test was positive.

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In this connection it should be mentioned that as early as in 1944 in his first description of Waldenström's macroglobulinaemia Waldenström ascribed these findings to hyperviscosity (9). Ott mentions in 1956 that the cal



Fig I Microscopical appearance of the tu mour removed from the skull Hematoxylin and eosin staining Magn 400×

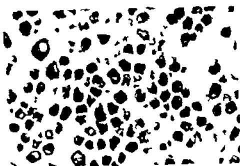


Fig II Plasmacell like tumour cells in the preauricular tumour Hematoxylin and eosin staining Magn 1000×

those plasmacells present showed a normal appearance definite increase in eosinophils and lymphocytes Erythrocyte sedimentation rate after one hour 5 mm

Serum proteins total protein 7 54 gr % (albumin 66 7 % alpha 1 globulin 2.8 % alpha 2 globuline 8 0 % beta globuline 9 5 % and gammaglobulin 13 0 %) With a rabbit anti human IgM serum, no precipitation line was seen in doubledimensional agar diffusion plate (Ouchterlony) with a dilution of the patient s serum 1 10

Serum value for alkaline phosphatase 18 2 units (King Armstrong) acid phosphatases 1 6 U (King Armstrong) thymol turbidity 0 7 U sodium 140 0 milliequivalents pro 1000 ml potassium 4 9 m equ. pro 1000 ml and chlo



Fig III X ray of the right knee showing the mottled appearance

ride 96 6 m equ pro 1000 ml Blood urea 40 mgr % creatin 0 58 mgr % with a clearance of 112 ml/minute *Rowntree 58 % (after two* hours) Weakly positive reaction for anti nuclear factors using an immuno fluorescent technique

Reactions of Weinberg Casoni and Von Pirquet negative VDRL negative

Urinanalysis was normal

No occult blood or worm eggs in the faeces

No clinical diagnosis could be made at this time The pre auricular tumour was removed and showed the unmistakable appearance of a plasmocytoma (fig II) Skin or bone tissue were not present in the tissue removed The tumour was divided into a few large lobules separated by broad bands of connective tis sue This distribution was suggestive of lym phoglandular involvement but as there was no normal lymphoid tissue present this can not be decided for with certainty

One year later the patient was admitted to another hospital for an infiltrate in the right lung due to a Staphylococcus aureus infec tion He recovered after treatment with peni cillin and streptomycin

About this period he started complaining about his right knee A progressive swelling developed in the right condyle of the femur

X rays of this area showed a mottled ap pearance (fig III)

2 months later biopsies were taken from this lesion and from another one in the right tibia Both were compatible with a diagnosis

Dept of Internal Medicine University Hospital Groningen (Prof E. Mandema) Dept of Pathology Groningen University (Prof A Arends)

A Patient with Atypical Multiple Myeloma

By A J VAN DER GRIENT and E J EBELS

The history of this patient relevant to the condition to be described started five years ago A man aged 52 years was admitted to the Department of Neurosurgery of this hospital with signs of herniation of the brain stem Although he had noticed a slowly increasing swelling of the cranium for some years he had been well until one month before admission At that time he started complaining of nightmares forgetfulness and headache He vomited once had fever up to 39 C and was sleepy One day before admission frequent vomiting started The patient was found to be drowsy and dehydrated At clinical examination the bloodpressure was normal there was no papilloedema and there were no signs of meningeal irritation At palpation a subcutaneous mass was present in the left frontal area which was continuous with or adherent to the skull X ray investigation showed bone destruction suggestive of tumour infiltration At operation (Prof C H Lenshoek) a golfball sized tumour was found adherent to the dura and infiltrating the overlying skull It was considered to be a meningeoma and removed as fully as possible At histological examination a cellular tumour was seen with more or less oval cells loosely arranged in fields separated by a mostly delicate stroma The nuclei were round sometimes excentrically situated and very often showed bizare clumping of chromatine (fig I) The possibility of an undifferentiated plasmocytoma was considered but rejected (partly on the ground of negative pyronin staining of the cytoplasm — it became apparent afterwards that our methyl green pyronin staining at that time was technically unreliable) The possibility of an atypical "cytoplastic meningeoma was suggested

The material was also seen by Prof Zulch in Cologne who suggested the possibility of a metastatic tumour a plasmocytoma and leucaemic infiltration We then asked Prof Dorothy Russell in London for her opinion She thought a plasmocytoma most likely but also considered the possibility of a reticulo sarcoma

Two years later a tumour developed in the region of the left mammary gland and it was removed surgically Histological examination showed a non encapsulated cellular tumour spreading within the fatty tissue of the mammary gland which itself showed the aspect of a mamma virilis the cellular aspect was similar to that of the first tumour but by now some unequivocal plasmacell like elements were present

Shortly afterwards a chestnut sized tumour developed in the left pre auricular region At this time the patient was investigated clinically in the Department of Medicine Physical examination was normal X rays of the bones did not show any abnormality Laboratory

Blood haemoglobin 14 8 gr % leucocytes 7800 (16 % eosinophils 29 % neutrophils 34 % lymphocytes some of which had the appearance of young types 1 monocytes) Sternal puncture no increase of plasmacells



Fig VI Supra inguinal tumour

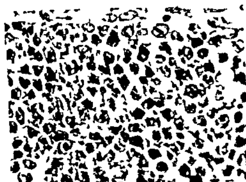


Fig VII Histological aspect of the supra inguinal tumour showing poorly differentiated cells Magn 700×

phoresis showed marked reduction of IgM and IgA globulins *with a paraprotein of the* IgG class On ultracentrifugation there was a marked increase of a 7 S globulin presumably caused by the IgG paraprotein An immunological search for IgG H chain paraproteinaemia was negative After injection of 5 mgr bromsulphalein per kg body weight 5 % was retained Urinalysis was normal No Bence Jones protein could be detected Again *no worm eggs were found in the faeces*

Bone marrow examination at this stage revealed out of 500 nucleated cells 41 (8 2 %) lymphoid plasmacells 3 (0 6 %) plasmacells 36 (7 2 %) eosinophilic leucocytes

The supra inguinal tumour was removed (fig VI) Histological examination showed a very cellular tumour with many features of those removed from the mammary and preauricular regions but with less differentiation of the cells into plasmacell like elements It was diagnosed as a poorly differentiated plasmocytoma (fig VII) on the same grounds as in the biopsy from the preauricular tumour the appearance of the tumour was suggestive of lymphoglandular involvement but here too this remained conjectural Skin was not present in the biopsy

In view of the multiple bone defects as seen on the X ray pictures the histological appearance of the tumours removed and the presence of an IgG paraprotein with a strong decrease of IgG and IgM globulins the diagnosis of multiple myeloma was made

The patient was treated with melphalan which caused some subjective improvement and some regression of the swelling *of* the knee The sedimentation rate of erythrocytes fell to 38 mm after one hour and 78 mm after two hours Six months afterwards a rapidly growing tumour developed in the right testis as well as infiltrates in the right upper leg and underneath the left eye Hemi castration was advised but was refused by the patient. X ray therapy caused some regression of the tumours The general condition gradually deteriorated and the patient died five years after the first admission Autopsy was refused

Discussion

The first symptoms of the present illness of this patient were those of involvement of the central nervous system due to a large poorly differentiated plasmocytoma which originated either from the skull or from the dura mater Later on multiple extra medullary plasmocytomas developed followed by the occurence of multiple bone lesions In the first stages serum protein values remained normal later on there was IgG para



Fig II X ray of the arms, showing lesions in the left ulna

of plasmocytoma (Prof J J Hampe University of Amsterdam)

As the patient s general condition deteriorated he was once again admitted to our department (about 4 years after the beginning of this history)

By now he was in a fairly bad condition Examination showed multiple haemangiomata of the skin Lymphnodes were not enlarged at palpation In the abdominal wall above the left inguinal region a chestnut sized tumour was present adherent to the underlying structures but not to the skin Liver and spleen were not enlarged (as they never were throughout the illness) X ray investigation showed extensive bone lesions to be present in the left tibia left ulna left radius right femur and right tibia (fig IV)

The blood pressure was 115/80 mm

Laboratory investigations

Blood haemoglobin 11 2 gr % Packed red blood cell volume 34 ml/100 ml erythrocyte sedimentation rate after one hour 115 after two hours 132 mm (Westergren) leucocytes 7300 (2 % basophils 16 % eosinophils 49 % neutrophils 27 % lymphocytes 6 % monocytes) At another occasion even 46 % of the leucocytes consisted of eosinophils! Thrombocytes 205 000 mm³ Analysis of blood clotting showed no abnormalities The tourniquet test

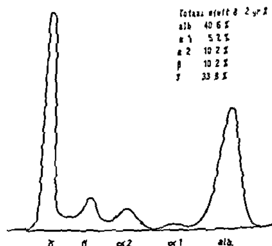


Fig V Electrophoretic pattern of serum proteins

was strongly positive Alkaline phosphatase 19 1 U (King Armstrong) Acid phosphatase 0 8 U (King Armstrong) Urea 35 mgr % Total bilirubin less than 1 mgr % Calcium 8 4 mgr % Phosphorus 2 6 mgr % Serum glutamate oxalate transaminase 3 U serum glutamate pyruvate transaminase 5 U Lactate dehydrogenase 175 U Total lipids 700 mgr % Serum proteins 8 12 gr % (of which albumin 40 6 % alpha 1 globulin 5 2 % alpha 2 globulin 10 2 % beta globulin 10 2 % and gamma globulin 33 8 %) (fig V) Immuno electro

to the small lymphocyte as being the precursor of the plasma cell (for a survey vide Gowans and MacGregor 8) In that case we must assume a local derangement of plasmacells or their immediate precursors as the initial event in the occurence of plasmocytomas As to extramedullary plasmocytomas the theoretical possibility of course remains of their being metastases of a bone lesion We think that there is little evidence in favour of such an explanation in our case

For the eosinophilia in this patient no ready explanation is available Unexplained eosinophilia occurs in rare cases of *multiple myeloma* Snapper et al (16) described three patients with respectively 20 % 32 % and 46 % eosinophils Mandema (13) in the *bone marrow examination of 36* patients found more than 10 % eosinophilic cells (as percentage of the nucleated cells of the bone marrow) *in 4 cases*

As long as the cause and nature of the eosinophilic reaction in general remain enigmatic every theory to explain the combination of multiple myeloma and eosinophilia must be speculative The more so as every explanation should at the same time *account for the occasional combination* and its very rarity We may perhaps consider eosinophilia to be an expression of some sort of antigenic *stimulation* In *multiple myeloma* either the hypothetical antigenic stimulus considered to be the (a) cause of the tumour process itself (for the literature vide Ten Berge, 18), or the *antigenic properties of the parapro*teins produced by the tumour may be factors that in some cases lead to eosinophilia as the expression of sensibilisation

Litterature

1 Batts M Jr., Arch Surg 39 807 1939
2 *Bodo Spielhoff* Arch f Dermatol und Syph 91 265 1908
3 Cutler Jr C W Ann Surg 100 392 1943
4 Duverne J et al., Soc Franc *Derm et* Syph 13 3 1958
5 Ewing J A, A treatise on tumors ed 4 Philadelphia W B Saunders Co 1940
6 Fayolle J., Thèse Lyon 1957
7 Gottron H A in Gottron H A and Schonfelt W Dermatologie und Venerologie IV *582 1960*
8 Gowans J L and MacGregor D D in Progress in Allergy IX Kallos P and *Waksman B H., Eds S* Karger Basel New York 1965
9 Hayes D W et al., *Arch Path* 53 262 1952
10 Hellwig C A Arch. Path 36 95 1943
11 Kindler U Dtsch med Wschr 90 1013 1965
12 Kreibich C Fol haemat 18 94 1914
13 Mandema E Thesis 1956 Groningen
14 Nelson M G *and Lyons A R* Cancer 10 1275 1957
15 Smith D Emslie et al J *Clin Path* 8 104 1955
16 Snapper A P et al., *Multiple Myeloma* Grune and Stratton New York (1953)
17 Stout, A P., et al J *Missouri Med* Ass 46 275 1949
18 *Ten Berge* B S Thesis 1965 Groningen
19 *Zanca, A et al* Fracostoro 57 404 1964

proteinaemia Throughout the illness unexplained eosinophilia was present The localisation of the bone lesions in the limbs, *especially distally*, is unusual

Batts (1) examined the distribution of bone lesions in 40 patients, in 24 by X ray investigation, in the other patients by other means He found the following distribution skull 73 %, vertebral column 70 %, ribs 68 %, pelvis 63 %, femur 48 %, shoulder and sternum 40 %

In our case the exact localisation of the extramedullary lesions from the pre auricular region and from the abdominal wall was suggestive of lymphoglandular origin, but this was not confirmed by the clinical impression The tumour from the mammary region was located in the fatty tissue of the gland

Localisation of plasmocytoma in the mammary gland was described by Cutler (3) and in the infra mammary region by Stour et al (17)

Localisation in lymphnodes and skin is exceptional Out of 153 extra medullary plasmocytomas collected by Ewing (5) 4 were situated in lymphnodes, none in the skin Hellwig (10) found lymphoglandular origin in 4 out of 128 extramedullary plasmocytomas and only 2 in the skin

Nelson and Lyons (14) collected 10 plasmocytomas located in lymphnodes Kindler in a very recent article (11) gives the following distribution of 207 extramedullary plasmocytomas

upper respiratory tract		137=66 1
gastrointestinal tract		25=12 1
lymphnodes	mediastinal	9= 4 4
	cervical	4= 1 9
	multiple	4= 1 9
	axillary	1= 0 6
lungs		10= 4 8
genital organs		5= 2 4
urinary tract		4= 1 9
endocrine organs		3= 1 4
skin		2= 1 0
hypothalamus		1= 0 5
lacrimal glands		1= 0 5
mamma		1= 0 5

Duverne (4) described a patie with a plasmocytoma of the skin i whom later on bone lesions develope

Apart from plasmocytoma oth skin lesions may be present in mu tiple myeloma, such as erythroderm abnormal pigmentation (especiall around the eyes), lesions due to circu latory disturbances and lesions con nected with amyloidosis (for a surve see Fayolle 6) An interesting condi tion is described by Gottron (7) as "plasmocytoma of the small reticulun cells of the skin" in which changes i the serum proteins are present simil to those of multiple myeloma!

Many authors even in recent artic les [Zanca et al (19) Kindler (11)] consider plasmocytomas to originate from the reticulo histiocytic system But most of them do not in their dis cussions take into account more re cent knowledge about the origin of the plasma cell And we feel that every theory in this field abuts on this very problem

Most evidence to day seems to point

1937 and the gel filtration 1959 each method has its disadvantage Due to the high lability of many biologically important bindings against changes in pH temperature and the ionic strength and/or the absence of possible competitive binding serum proteins in the isolated fraction all precipitation methods for investigations of bindings are to be looked at very critically

Before we go further into the transport processes of bilirubin we will briefly characterize the transport milieu in which bilirubin itself moves Under "*transport milieu*" (15 16) is meant that mobile milieu in which substances and biological units are contained and which through transitory bindings serve the transport processes as such The substances to be conveyed in the blood totally or partly bound to serum protein or to erythrocyte vehicles can be collected together under the heading of "*transport goods*" In doubtful cases it is possible to decide by the determination of the biological half life which of the complexes circulating with the blood should be regarded as the vehicles and which is the transport goods (compare the studies of Laurell (55) on iron bindings) The vehicles then are the components of the transport milieu which always have the longer biological half life

Labile equilibrium conditions often arise between the same materials which are bound to different protein vehicles The very size of all vehicles in the blood are completely different the erythrocytes with diameter of 7μ the thrombocytes with 2μ diameter then the serum proteins following (in order of size) can only be compared with one another by their molecular weights (m w) alpha₁ lipoproteins, with for example a m w of 3 400 000 then transferrin with a m w of 88 000 albumin m w 69 000 and the alpha₁ glycoproteins with a m w 50 000 down to 44 000

For the transport processes the really important factor — that of surface area — amounts in the erythrocytes for an adult to 3 200 sq m , and approximately at least 200 times this for the serum proteins The vehicle apparatus has to maintain the cells in the human body the number of which is of the order of 10—100 billion (10^{13}—10^{14}) (24) The total length of the capillaries only in the musculature of a normal adult is according to Krogh (51) 100 000 kms The erythrocytes must not only be considered as transporters of oxygen from the lungs to the tissue and as removers of carbon dioxide from the venous capillaries and back to the lungs

The functional analogy between the corpuscular erythrocytes and the colloidal serum proteins is supported by phylogenetic observations the earthworms and some crabs use instead of erythrocytes a chromoproteid sol with a m w of some millions the function of oxygen containing vehicles is fulfilled in these animals by the colloids erythrocruorines and chlorocruorines —

It is known that in the human body numerous medicaments such as sulphonamides and mepacrin (98 14 17) but more particularly many hormones form supplementary bindings with the erythrocytes The most carefully

The Transport of Bilirubin in the Circulating Blood and Its Pathogenetic Importance

By H Bennhold

Bilirubin is probably the first indigenous substance in the body, in which it was possible to demonstrate the binding to serum proteins This was done originally by ultrafiltration or dialysis of bilirubin containing serum (73, 74, 32) By electrodialysis and by kataphoresis Bennhold (9) discovered a special albumin bilirubin binding and based on this observation and on experiments with dyes and cholesterole (11) the conception of a general "vehiclefunction (9 12 13 14 18) Bendien and Snapper (7) in 1933 also found bilirubin mainly bound to albumin, by using a graduated permeable ultrafilter Pedersen and Waldenstrom (70) confirmed very reliably in 1937 this albumin binding by electrophoresis and for the first time by the ultracentrifuge

In recent years the exclusive binding of bilirubin C^{14} to the albumins at least regarding transport processes was proved by free two dimensional electrophoresis (67) The older notion of plasma protein as a waste product stream (45) was not attractive to physiological research Even Krehl (49) in his "Pathologische Physiologie' 1930 p 471, put forward the same supposition It was purely from the degree of precipitation with ammonium sulphate that it was possible at that time to distinguish between albumin globulin and pseudoglobulin — Only cellular components of the circulating blood such as erythrocytes and leucocytes were credited with a transport function but not these three waste serum protein fractions, although as early as 1896 Starling had shown the hydrophilic affinity of the serum proteins and its importance for the pathways of the water in capillaries and in the tissue

Since then the number of separated serum proteins obtained by different methods (26 37 90 93 94) has risen to approximately 100 while the total number of substances including hormones and medicaments which are bound to serum proteins is now no longer surveyable The most careful methods of proving bindings to serum proteins were the ultrafiltration and dialysis 1925 the electrophoresis (8 12 93) (1928—1932) the ultracentrifuge

1937 and the gel filtration 1959 each method has its disadvantage Due to the high lability of many biologically important bindings against changes in pH temperature and the ionic strength and/or the absence of possible competitive binding serum proteins in the isolated fraction all precipitation methods for investigations of bindings are to be looked at very critically

Before we go further into the transport processes of bilirubin we will briefly characterize the transport milieu in which bilirubin itself moves Under "*transport milieu*" (15 36) is meant that mobile milieu in which substances and biological units are contained and which through transitory bindings serve the transport processes as such The substances to be conveyed in the blood totally or partly bound to serum protein or to erythrocyte vehicles can be collected together under the heading of "*transport goods*" In doubtful cases it is possible to decide by the determination of the biological half life which of the complexes circulating with the blood should be regarded as the vehicles and which is the transport goods (compare the studies of Laurell (35) on iron bindings) The vehicles then are the components of the transport milieu which always have the longer biological half life

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It is known that in the human body numerous medicaments such as sulphonamides and mepacrin (98 14 17) but more particularly many hormones form supplementary bindings with the erythrocytes The most carefully

studied of such binding equilibria are the bindings of thyroid hormones to serum proteins and erythrocytes Thyroxine thus adheres to interalphaglobulin, to prealbumin (1) *and albumin itself (35, 61), but only loosely* to erythrocytes (66) *Similarly triiodothyronine* is bound to *interalphaglobulin* and albumin, but its binding *affinity* to the erythrocytes is four times *greater than that of thyroxine* The adrenal hormone cortisol *is bound to* at least three components *of the transport* milieu, in the first place, approx 90 %, to transcortin (28, 30), an alpha glycoprotein, which occurs normally only in a *concentration of* 2 3—4 6 mg % (30), there is in addition *though,* a considerably weaker binding to albumin, and finally an even *weaker binding to the* erythrocytes *(63) The* binding to transcortin is *temperature labile*, decreasing with increasing temperature (measured at *1°, 37°, and 60°)*, while the binding *to albumin* is temperature stabile (85)

Quite how does bilirubin fit into this *complicated transport* milieu, in spite of its concentration in certain illnesses being increased to upto 50—100 times the norm?

Firstly though in order to prevent errors, two totally different transport types should be differentiated in the living organism The first is the long-distance transport from organ to organ — 'the macro distance transport — the motive power of which is caused directly by the pumping action of the heart and takes place in a clearly visible mobile transport milieu the blood

The second *form of transport* travels *only* over very *short distances* — *"micro distance transport"* — *for the most part within fixed* membranes, or within the cells themselves The diffusion processes belong to this type, but even more important, the "active transports", which by the insertion of *the* carrier (e g ATP) require chemical energy One would also include the operations of ultrafiltration and colloidosmosis in the micro distance *transport* phenomena, although they too are in fact partly driven by motive power from the heart

Both types of transport processes can directly succeed *one another The* change over position usually occurs at the point of contact of the circulating blood stream or the tissue liquid with the membrane of the cell or other tissue components The transport milieu of the circulating blood guarantees therefore only the 'form of offering" of the transport goods at the surface of the fixed tissue —

Similar exchange processes between binding to albumin and binding to certain tissue elements (amyloid) can occur rapidly even when no enzymatical influence can be supported

1) The relationship between the disappearance rate of the Congo red — normally albumin bound — and the albumin level in the blood was especially evident in the two analbuminaemia patients it amounted to 7? *and 92 %* respectively in one hour instead *of* the normal 15—29 % — With nothing but 202 g albumin given intravenously the disappearance rate was normalized (20 69)

2) The high affinity of Congo red to the amyloid substance in vitro is known since 1922 (8a 9a) It therefore is probable that in patients with amyloidosis the speed of disappearance of the dye is mainly caused by

the higher binding affinity of Congo red to the amyloid substance than to the albumins By comparing simultaneously the Congo red concentration in the vena cubitalis and in the vena hepatica Lanai (72) showed in 9 patients with extensive amyloidosis of the liver a difference of 12.2 to 47 9 % within one passage through the liver

The literature concerned with these processes has grown enormously from 1938 to the present day Lit Bennhold and Ott (20) Hitzig (46) de Traverse (95) Schwick (84)

Before we continue and consider the particular characteristics of bilirubin transport we must first explain the meanings of "*Insufficiency of Transport Milieu*" and "*Transport Illnesses*" with selected examples An "insufficiency of transport milieu" manifests itself clinically in that in fixed tissue *adhesion points of second rate affinities* will be occupied or additionally occupied by the transport goods in question normally fixed to the serum proteins "Transport illnesses" are illnesses where a detectable insufficiency of one or more components of the transport milieu plays a deciding pathogenetic role An insufficiency of the transport milieu can be caused by the following conditions

1) A congenital deficiency or even a fault in the development of one of the components of the "vehicle apparatus" (for example in the field of the erythrocytes sickle cell anaemia thalassaemia and the atypical hereditary haemolytic anaemias besides the genetic methaemoglobinaemia on the field of serum proteins analbuminaemia (6 24 36 68) atransferrinaemia (44) and deficiency of al pha antitrypsin (56) and other defectpathoproteinaemias

2) Acquired toxic, or other similar quantitative or qualitative damages to the vehicles CO poisoning toxic methaemoglobinaemia (as for example in nitrous poisoning), toxic anaemia iron deficiency anaemia, (damaged oxygen transport), dysproteinaemia and hypoproteinaemia *e g protein* loosing gastroenteropathia, Kwashiorkor (72) and nephrosis (58)

3) A pathological increased influx of certain transport goods into the blood without a corresponding possibility of adjustment of the vehicle in question *this is the case in primary* haemochromatosis (15 43 99) where an uncontrolled resorption of iron in the intestine results in a permanently increased iron influx into the blood, without a corresponding increase of the transferrin level

4) A pathological blockage in the *utilization of the transport goods* so that a reduction in the physiological removal and thereby a pathological accumulation of transport goods in the blood stream occurs without sufficient compensation possibilities being activated An example of this is *Wilson s disease* (15) which through the blocking of the ceruloplasmin synthesis and by an excessive absorption of copper in the intestine leads to pathological accumulation in the blood of the only loosely albumin bound unutilized copper (76 77 92), this results in an occupation of the secondary binding sites in the tissue whereby the *symp*tomatology of the illness is characterized (15 16)

Analogous to this is the *sideroachrestic anaemia* (42), which often results in haemochromatosis, because the blockage of the haem synthesis causes an accumulation of *iron in* the blood, and an insufficiency of the available transferrin

5) *A decompensated* transport milieu, through an influx of foreign substances, which by competition displace bound substances from sites on the vehicles This is of considerable importance, ***for only the components of medicaments (23) and hormones (2) not bound to the serum proteins, are pharmacologically and endocrinologically active*** The *protein bound* components are thus in inactive reserve A diabetic with well adjusted tolbutamide medication can suddenly develop severe hypoglycaemia, when given *sulphon amide* simultaneously Both medicaments occupy the same binding sites on albumin (17 14 29 83 98), which results in competitive displacement reactions and in increasing the concentration of free active tolbutamid in the blood (23) Conversely in the test of albumin bound bromsulphalein (BSP) tolbutamide accelerates the disappearance of the dye from the blood in the liver and can therefore in liver tests lead to incorrect diagnosis (38 39) In addition to this though tolbutamide can, through a similar substitution, sink a pathologically increased bilirubin level through displacement of bilirubin into the tissue (64) Brodie (23) has explained the antirheumatic effect of the salicylates of phenylbutazone and of indomethacin on the basis of the displacement of the endogenous cortisole from its binding to the albumins

The *situation* of the bilirubin is exceptionally complicated regarding macro- and micro distance transports.

Bilirubin results from the autonomous haemoglobin metabolism as an intermediate product Apart from this, as soon as it *penetrates into cells* other than liver cells, it produces most serious injuries to the cell respiration by uncoupling the enzymatical oxidative phosphorylation (100) The transport of this highly toxic material from organ to organ must be particularly well regulated

1 mol of albumin binds with 2 mol of non conjugated bilirubin (67) With bilirubin C^{14} it was proved in continuous flow electrophoresis that without doubt only albumin is concerned as a binder in the macro distance transports of bilirubin Even in a case of a child with congenital non haemolytic jaundice lacking glucuronyl transferase in the microsomes of the liver cells (Crigler Najjar disease), it was found that in electrophoresis of the serum, inspite of a bilirubin level of 22 mg % the curves of the albumins and the bilirubin C^{14} coincided very closely (67)

Special transport problems arise in the foetal circulation

Schenker Dowber and R Schmid (78) made the following observations in guinea pigs 2 hours after the injection of unconjugated bilirubin C^{14} into the umbical, 52—66 % of the radio activity was found in the bile of the mother, after the injection of conjugated bilirubin though only

4 % The same results were found in apes (57) — If the foetus of the guinea pigs were injected with unconjugated bilirubin and at the same time additionally with albumin I^{131} only the bilirubin passed the placenta but not the previously binding albumin I^{131} The foetal bilirubin must therefore come into contact with particularly strong binding elements of the placenta which loosen the in vitro stable bilirubin albumin binding the dissociated bilirubin can then pass over into the circulation of the mother

The erythrocytes are also able to bind small quantities of unconjugated bilirubin to their lipoid membranes In vitro 100 ml of washed and packed erythrocytes can reduce by absorption the bilirubin level of a hyperbilirubinaemia serum by 3 mg % Added albumin brings about a equilibrium between bilirubin bound to erythrocytes and to albumin (96) With Coomb s positive erythrocytes the binding affinity of bilirubin as compared with Coomb s negative erythrocytes is reduced by a half This can have some significance in the interchange transfusion with erythroblastose children the exchange of the Coomb s positive erythrocytes of the baby for the Coomb s negative erythrocytes of the donator can almost double the rate of elimination of the bilirubin by the erythrocytes —

Because not only the indirectly reacting but also the directly reacting glucuronized bilirubin is normally bound quantitatively to serum protein every jaundice must be a clinical symptom of an insufficiency of transport milieu for bilirubin The localization of bilirubin in the tissue results from the secondary or tertiary affinities of elastica *in the sclera and* in the skin of the cartilage and osteoid tissue

Which of the five previously outlined conditions for an insufficiency of the transport milieu concerning bilirubin are now realized by the different forms of jaundice?

I *Deficiency of certain vehicles* In the case of missing or almost completely missing vehicles for bilirubin e g in analbuminaemia (19 21 36 59 60) we find surprisingly enough no truly clinical symptoms of an actual insufficiency of the transport milieu for this highly poisonous bilirubin no jaundice nor Kernicterus! — Perhaps the overloaded scarce albumin vehicles *bind the bilirubin more* loosely which leads to an accelerated delivery onto the liver cells like the displacing effect of tolbutamid for bilirubin bound by albumin mentioned above (p 168) It is altogether surprising that in analbuminaemia in *spite of the very multiple* although of course labile binding capacity of the albumins in healthy people usually very few clinical symptoms appear Even oedema is not obligatory Thus it is obvious that in this recessive hereditary anomaly various compensatory *mechanisms intervene* in the various organs (19 59 60 69 73) In the other cases of congenital or acquired deficiency (e g nephrosis (58) no jaundice is observed It is remarkable that the loss of 95 % of circulating albumins with such a multiplicity of

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defect in Gunn rats The under developed glucuronization apparatus in premature babies is an essential factor in the outbreak of a serious illness the Kernicterus *In some pregnancies* hormones are developed which in the foetus and also in the *infant during* breast feeding impedes the glucuronyl transferase (33 54) in this way serious jaundice and eventually kernicterus can occur (3 4 a) The antibiotic novobiocin similarly impedes the glucuronization and so does chloromycetin (50) The excretion hindrance of the *glucuronized bilirubin* is interpreted as the cause of the congenital Dubin Johnson syndrome *(31)* and also of the Rotor syndrome (75) Also jaundice after chlorpromacin is supposed to result from an excretion *hindrance in the cell membrane Billing* (22) injected bilirubin intravenously *(2 mg/kg body weight) to 7 normal* subjects and 22 patients with conju*gated and unconjugated hyperbiliru*binaemia The curves were fitted by 2 *exponential terms In numerous* cases she could localize the damage either *on the membranes* where *bilirubin en*ters or where it leaves the cells or in the *microsomes The further transfor*mation and the pathway of bilirubin in the bile ducts in the *intestine and* in the enterohepatic circle are not within the scope of this paper In the *lymph which has a relieving role in* bile obstruction bilirubin is bound to the albumins too (97)

Decompensated transport milieu by influx of competing substances In transports with temporary binding to proteins obviously displacement processes frequently play an important role These can be localized either in the circulating plasma proteins or intracellularly in the organs which carry out the further treatment or elimination It is obvious that *combinations of both types are possible* The localization of these displacement processes can be ascertained through the determination of the disappearance *rates first by intravenous application* of each single competing substance *and then by simultaneous application* If the competition has its seat solely *in the plasma* that is to say within the sphere of the macro distance trans*ports the displaced material produces* by occupation of the binding sites on *the serum proteins* a sudden decrease in the concentration of the removed *material* Examples are the accelerated disappearance of intravenously injected bilirubin C^{14} by additional injection of salicylate (82) acceleration *of the disappearance curve* of brom sulphalein through addition of tol*bulamide* carbutamide or sulphonamides (44) — If however the place of *competition lies within the domain of* the admitting livercells or the elimina*tion organs* thus within the sphere of the micro distance transport then the *displaced material produces an elimi*nation hindrance from the blood *stream* this leads to a reduction in the concentration decrease or even a flow back *into the vessels Examples* of this are a check in the BSP disappearance curve through the *simulta*neous application of rose bengal (25, 62) *of bilirubin (64)* or *of sodium* dehydrocholate (53) It is only possible

bindings to the transport goods, is so well compensated by the organism Only if certain exceptional transport loading occurs, e g through intravenous injection of medicaments usually bound to albumins, serious complications can arise (17) — In contrast to this though in *atransferrinaemia*, the almost complete deficiency of the — normally 6 6 g — circulating transferrin, has serious clinical consequences (44) There appear everywhere in the different organs serious damages through pathological iron deposits, because of the failure of the specific transport milieu without compensating mechanisms In the analbuminaemia cases we found a low bilirubin level (only traces) Will this reduced quantity of bilirubin be still adequately bound to the circulating albumin?

II *Qualitatively damaged vehicles* We do not know of any toxic influence which might modify the albumin qualitatively, so that its binding capacity pro g albumin would be decreased (analogous to carboxy haemoglobin in the erythrocytes)

III *A pathologically increased influx of bilirubin* appears in all illnesses with a clearly increased decomposition of the erythrocytes It can be genetically conditioned, as for example in the constitutional haemolytic jaundice, in the hereditary non spherocytic anaemia, in thalassaemia, in sickle cell anaemia, and finally to a certain degree, in pernicious anaemia

The macro distance bilirubin transport by the albumins is — in spite of the extensive bilirubin production — in most cases capable to avoid bilirubin intoxications In illnesses mentioned above the macro distance transports taking place in the vascular system, are relieved through the joining of the intrahepatic, micro distance transports and through the large reserve of the intracellular treatment apparatus According to Sherlock (86, 87), the bilirubin content of the blood increases for example by only 2—3 mg %, while through haemolytically active poison, the influx of bilirubin rises to 1500 mgm per day, that is to six times of the normal

IV *A pathological blockage to the further treatment of the transport goods* bilirubin is found under following circumstances on the way into and through the livercells, bilirubin has three important stations to pass 1) the membrane, 2) the microsome with the enzymes for the glucuronization, 3) the exit point of the glucuronized bilirubin through the cell membrane into the bile ducts Sherlock and Billing (87, 22) have collected descriptions of certain illnesses with damages of the cell membrane, as well as of differentiable organelles inside the livercells In the congenital Gilbert's illness (34) the entry of the bilirubin into the livercells through the cell membrane is impeded It also appears that maleferm extracts are able to hamper the admission of bilirubin into the livercells

In the second stage the microsomes the glucuronization of the bilirubin takes place Congenital enzyme defects e g Crigler Najjar syndrome (27) occurs in the microsomes This correponds exactly with the enzyme

treatment with salicylate or sulphonamides (81).

Odell was the first to draw therapeutic conclusions from these clinical observations and experiments by albumin infusions. He succeeded in displacing bilirubin out of the tissue. These albumin infusions have particular value when they are connected 30 minutes later with an exchange transfusion, so that part of the bilirubin drawn out of the tissue into the blood stream can be removed. Koch and Rind (48) have injected intravenously selected cases of premature babies with serious hyperbilirubinaemia with 1 g of albumin per kg body weight; after a "reflux" time of 30 minutes they attached an exchange transfusion. The largest quantity of eliminated bilirubin could in one exchange amount to 168 mg. The number of cases where second and third exchange transfusions were necessary could be decreased by this treatment from 25 % to 7 %.

From these examples of kernicterus we can see the damage to the total organism which can arise when the transport milieu becomes insufficient for such toxical transport goods as bilirubin. We recognize further what great significance the displacement processes can have for the micro-distance transports. — Also in vitro presence of bilirubin considerably limits the binding capacity of albumin for sulphonamides (83). — A hyperbilirubinaemia just as severe hypoalbuminaemia can under certain circumstances disturb the optimal peripheral distribution of medicaments and the delivery to the tissue and the organs. This refers particularly to intravenously applied medicaments in which the speed of injection plays an important role in the initial delivery of transport goods to the tissue.

If unexpected side effects of medicaments occur, the clinician should consider the possibility of a transport insufficiency. To exclude such factors, however, not only the absolute albumin contents of the blood should be determined, but also competition processes by other albumin bound material must be taken into account.

With the complexity of the transport milieu in the blood, it should be considered whether the illnesses which are decisively conditioned by insufficiencies of the vehicle functions should not be withdrawn from the mass of metabolic illnesses under the heading of "Transport Illnesses"; this might be also be of value for the therapeutic perspective.

Summary

1. A brief survey has been given about the development of the opinions of the bilirubin bindings to the serum proteins. The albumin bindings for this highly toxic substance are of the greatest importance for bilirubin transport.

2. The idea of "transport milieu" and its insufficiency symptoms have been interpreted from some illnesses.

3. A difference has been bade between the macro distance transports in the blood stream and the micro di

to surmise the localization of these competition processes, particularly within the livercells These considerations will concern the transports of bilirubin through the cell membrane to the microsomes and through the excreting membranes to the bile ducts It is possible that sodium desoxylate impedes not only the elimination of the BSP from the blood, but, additionally, after the fading away of the desoxycholine, the dye flows back into the bloodstream This suggests that the hindrance to further treatment of the bilirubin and also of BSP in this case, is localized near the entrance to the cell membrane In Dubin-Johnson disease this reflux of the dye into the *bloodstream was exceptionally high*

The processes on the cell membrane, and in the intracellular transports as far as to the microsomes can however after the previously described experiments, be explained with a considerable degree of probability as a consequence of a physico chemical binding gradient [adsorption chain according to Kallee (47)] in a similar way Paller (71) has advanced suggestions about binding colloids on both sides of the cell membrane

In the dramatic illness of Kernicterus, based on erythroblastosis in premature babies we find all the five previously *mentioned* causes of serious insufficiency of transport milieu combined in the same illness

I) The albumins as the vehicles for bilirubin are reduced compared with the full term babies

II) The Coomb s positive erythrocytes of these patients bind only half is much bilirubin as the Coomb s negative would normally do

III) The large amount of bilirubin caused by the extensive haemolytic processes results in a very high bilirubin level in the blood

IV) The microsomes in the liver cells and their glucuronyl transferase production are still not completely developed, so that the normal way of elimination for the glucuronized, now water soluble, bilirubin through the kidneys cannot be used

V) The children are also endangered by the series of medicaments which should be used because of their general under development and diminished resistance These drugs often *occupy the same binding sites* as bilirubin (82 83) In this way actual competition processes arise the lipid soluble bilirubin bound to the albumins is displaced from these binding sites and finds new binding sites on the elastica of the tissue with newborn children, however this can take place also on the lipids of the central nervous system (65)

These medicaments are normally harmless remedies such as salicylate sulphonamide coffein benzoate and antibiotics etc It was Silverman (89) who first established that after premature icteric children had been treated *with such medicaments* the percentage of those with Kernicterus rose sixfold

In young Gunn rats with their genetically conditioned deficiency of the glucuronyl transferase and their hyperbilirubinaemia similar cerebral damage can be produced through

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Summary

1 A brief survey has been given about the development of the opinions of the bilirubin bindings to the serum proteins The albumin bindings for this highly toxic substance are of the greatest importance for bilirubin transport

2 The idea of "transport milieu" and its insufficiency symptoms have been interpreted from some illnesses

3 A difference has been bade between the macro distance transports in the blood stream and the micro di

stance transports, which to a large extent take place intercellularly The two types of transport often succeed each other The albumins with their variety of binding abilities, and thereby the frequent opportunities for displacement processes have been discussed

5 The five most frequent causes of an insufficiency of a transport milieu have been described

6 These have been collectively demonstrated using the example of Kernicterus

References

1 Aly F W und K H Gillich Protid Biol Fluids Proceed 8th Collq Bruges 1960 152

2 Antoniades H N Hormones in Human Plasma Little Brown & Comp Boston 1960

3 Arias I M and S Wolfson Gastroenterology *38* 797 (1960)

4 Arias I M and L M Gartner Nature *203* 1292 (1964)

5 Arias I M Wolfson S Lucey J F and Mc Kay R J jr J of Clin Invest *44* 1442 (1965)

6 Beck G L et Dorta I Helv med Acta *26* 764 (1959)

7 Bendien W M and Snapper I Bioch Z *260* 105 (1933) Bioch Z *261* 1 (1933)

8 Bennhold H Verh dtsch ges inn Med *40* 455 (1928)

8a Bennhold H Munch Med Wschr

9 Bennhold H Verh dtsch Ges inn Med *41* 211 (1929)

9a Bennhold H Dtsch Arch Klin Med 142 32 (1923)

10 Bennhold H Verh dtsch Ges inn Med *42* 353 (1930)

11 Bennhold H Verh dtsch Ges inn Med *43* 211 (1931)

12 Bennhold H Erg inn Med Kinder heilk *42* 273 (1932)

13 Bennhold H Klin Wschr *11* 2054 (1932)

14 Bennhold H Kylin, E und Rusznyak St Die Eiweißkörper des Blutplasmas Verlag Theodor Steinkopff Dresden Leipzig 1938

15 Bennhold H Klin Wschr *38* 345 (1960)

16 Bennhold H Klin Wschr *41* 109 (1963)

17 Bennhold H Antibiotica et Chemotherapia *12*, 1 (1964) Verlag Karger Basel — New York

18 Bennhold H V Sympos Ouest Européen de Chimie Clinique 1965 In press Elsevier Publishing Comp Amsterdam — London — New York

19 Bennhold H D Klaus und P G Scheurlen Lancet 1960 *II* 1169

20 Bennhold H und H Ott In Handb d allg Pathologie von Buchner Letterer u Roulet Bd *V I* 166 ff Springer Verlag Berlin Göttingen Heidelberg 1961

21 Bennhold H H Peters und E Roth Verh dtsch Ges inn Med *60* 630 (1954)

22 Billing B H R Williams and T G Richards Clin Sci *27*(2) 245 (1964)

23 Brodie, B B V Sympos Ouest Européen de Chimie Clinique 1965 Elsevier Publishing Comp Amsterdam — London — New York

24 Bucher O Histologie und mikroskopische Anatomie des Menschen Med Verlag H Huber Bern — Stuttgart 196?

25 Cohen S J E Giansiracusa L A Strait T L Althausen and S Karg Gastroenterology *25* 232 (1953)

26 Cohn E J Strong L E Hugues, W L jr Mulford D J Ashworth J Melin M and H L Taylor J Amer chem *68* 459 (1946)

27 Crigler J F jr and V A Najjar Amer J Dis Child *83* 259 (1952)

28 Daughaday W H and I Kozak Maritz Metabolism *10* 11 (1961)

29 Davis D B Science *95* 78 (1942)

30 Doe R P J of Clin Endocrinol and Metabolism *24* 10 (1964)

31 Dubin I N and Johnson F B Medecine *33* 155 (1954)

32 Forrai E and R Sivo Biochem Z *189* 162 (1927)
33 Gartner L M Arias T M J Pediatr 65 1045 (1964)
34 *Gilbert, A et P Lereboullet Sem méd 21* 241 (1901)
35 Gordon A H J *Gross D O Connor* and Pitt Rivers R Nature (London) *169* 19 (1952)
36 Gordon jr R S., Bartter F G and Waldmann T Ann intern Med. *51* 553 (1959)
37 Grabar P et C A Williams Biochim. biophys Acta *10* 193 (1953)
38 Hasselblatt, A und T Hukuhara Klin Wschr *42* 449 (1964)
39 Hasselblatt, A T Hukuhara und J *Ziegler Klin Wschr 43* 220 (1965)
40 Hasselblatt A Klin Wschr *43* 913 (1965)
41 Heilmeyer L Acta haemat *23* 1 (1960)
42 Heilmeyer L Fol haemat. N F 6 9 (1961)
43 Heilmeyer L Folia haemat. *8* 1 (1963)
44 Heilmeyer L W Keller O Vivell W Keiderling K Betke F Wohler u H E Schultze Dtsch med Wschr 1745 (1961)
45 Herzfeld E und Klinger R Biochem Z *83* 42 228 (1917)
46 Hitzig W H Die Plasmaproteine in der klinischen Medizin Verlag Springer Berlin Gottingen Heidelberg 1963
47 Kallee E u W *Oppermann Z* Naturforschg 13 b 532 (1958)
48 Koch Fr *und H Rind Dtsch med* Wschr *90* 1358 (1965)
49 Krebl L Pathologische Physiologie I Bd 13 Aufl 471 (1930) Verlag F C.W Vogel Leipzig
50 Kretschmer N Pediatrics *23* 638 (1959)
51 Krogh A Introductory paper Animal Membranes Trans Faraday Soc. *33* 912 (193)
52 Lanari A A Patalano u A Soubrié Dtsch med Wschr 730 (1951)
53 Lang N Klin Wschr *37* 331 (1959)
54 Lathe G H and M Walker Quart. J exper Physiol. *43* 257 (1958)
55 Laurell C B In Der Eisenstoffwechsel, S 103 Edit W *Keiderling Thieme* Verlag Stuttgart 1959
56 Laurell C B and S Eriksson Verh dtsch Ges inn Med *70* 537 (1964)
57 Lester R R E Behrmann and J F Lucey Pediatrics *32* 416 (1963)
58 Linneweh F D Dtsch med Wschr 927 (1951)
59 Lohss F und E Kallee In H Peeters (Ed) *Protides of the Biological Fluids* Proc 8th Coll Bruges 1960 Elsevier Amsterdam 1961 (p 142)
60 Lohss F und H Ott Dtsch Arch. Klin Med *206* 426 (1960)
61 Maurer W und L Reichenbach Naturwiss *39* 261 (1952)
62 Mendeloff A. I. P Kramer F J Ingelfinger und S E Bradley Gastroenterology *13* 222 (1949)
63 Migeon C J B Lawrence J Bertrand, *and G H Holman J of clin Endocrin.* Metabolism Vol XIX, No *11* 1411 (1959)
64 Myers J D Proc 41 Meet Amer Soc. Clin Invest J Clin Invest *28* 801 (1949)
65 Odell G B J Clin Invest. *38* 823 (1959)
66 Olichney M J., K R Crispell and J D Harrah Acta Endocrinologica *48* 473 (1965)
67 Ostrow J D and R *Schmid J of Clin* Invest. 42 1286 (1963)
68 Oski F A *and J L Naiman J Pediatr* 63 1034 (1963)
69 *Ott, H Z* ges exp Med *128* *340* (1957)
70 Pedersen K. O und J Waldenstrom Z physiol. Chem 245 152 (1937)
71 Piller M Schweiz med Wschr 1963 p 1034
72 Purves, L. R. and J D L. Hansen South African Med J 36 1047 (1962)
73 Rosenthal S M J of Pharmacol *XIX* 385 (1922)
74 Rosenthal S M and E. C White J.A M.A *84* 1112 (1925)
75 Rotor A B., L. Manahan and A *Florentin* Acta med Philipp 5 37 (1948)
76 Scheinberg J *H and I. Sternlieb Gastroenterology 37* 550 (1959)

77 Scheinberg J H and I Sternlieb J Med clin N Amer 44 665 (1960)
78 Schenker St N H Dawber and R Schmid J of Clin Invest 43 32 (1964)
79 Scheurlen P G and D Klaus Klin Wschr 38, 123 (1960)
80 Schmid R S Burkingham G Mandilla and I Hammaker Nature 183 1823 (1953)
81 Schmid, R, J Diamond L Hammaker and C B Gunderson Nature 206 4988 (1965)
82 Schmid R J Diamond L Hammaker and C B Gunderson Nature 206 4988 (1965)
83 Scholtan W Arzneimittelforschg 11 707 (1961)
84 Schwick H G Therapiewoche 21 1127 (1965)
85 Seal U S and R P Doe Endocrinology 73, 371 (1963)
86 Sherlock S Brit med J 1359 (1962)
87 Sherlock S Krankheiten der Leber und der Gallenwege 3 Aufl J F Lehmanns Verlag Munchen 1965
88 Shetlar M R R W Payne G Stidworthy and D Mock Ann intern Med 51 1379 (1959)
89 Silverman W A D H Andersen, W H. Blanc and D N Crozier Pediatr 18 614 (1956)
90 Smithies O Biochem J 61 629 (1955)
91 Starling E H J physiol Lond. 19 629 (1955)
92 Sternlieb I and J H Scheinberg J of Amer med Assoc 183 747 (1963)
93 Theorell H Biochem 7 223 1–3 (1930)
94 Tiselius A Nova Acta Soc Sci Upsal Vol IV 7 1 (1930)
95 de Traverse P A Sympos Ouest Europeen de Chimie Clinique Avril 1965 in press Elsevier Amsterdam — London — New York
96 Watson D Clin Chim Acta 7 733 (1962)
97 With T K Biologie der Gallenfarbstoffe G Thieme Verlag Stuttgart 1960
98 Witzgall H und H Boyens Arch Internat Pharmacodynamie et de Therapie Bd 148 S 511 (1964)
99 Wöhler F Verh dtsch Ges inn Med 300 (1964)
100 Zetterström R and L Ernster Nature 178 1335 (1956)

III

BLOOD DISEASES

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Siderocytes, Sideroblasts and Sideroblastic Anaemia

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Siderotic granules in erythrocytes demonstrable by Perls's Prussian blue reaction were first described by Gruneberg (17 18) in the erythrocytes of mouse rat and human embryos and in the erythrocytes and erythroblasts of mice with a congenital anaemia (19) They were soon found however to be present sometimes in the erythrocytes of human patients who had undergone splenectomy (13) Grüneberg called these cells siderocytes

Dacie and Doniach (9) in a study of several patients with severe blood diseases i e congenital and acquired haemolytic anaemias after splenectomy and myelosclerosis showed that similar iron containing granules were present in the haemoglobinated erythroblasts of patients whose peripheral blood contained many siderocytes They confirmed too that many of the discrete basophilic granules demonstrable by Romanowsky dyes which might be seen in the erythrocytes of patients after (for instance) splenectomy — the so called Pappenheimer bodies (30)— stained positively for iron

Subsequently, Douglas and Dacie (14) and Kaplan Zuelzer and Mouriquand (23) studied in detail the occurrence of siderotic granules in the erythrocytes and their nucleated precursors in normal subjects and in a wide range of blood disorders Siderocytes were not found in the peripheral blood in health although they were demonstrated in marrow reticulocytes they were found sometimes in large numbers after splenectomy Siderotic granules were however demonstrable in from 20—90 % of the erythroblasts in normal subjects the granules were always small and sometimes difficult to see They were also found in erythroblasts in a wide range of blood diseases they were always absent however where there was iron deficiency

Kaplan Zuelzer and Mouriquand (23) introduced the term "sideroblast" to describe an erythroblast in the cytoplasm of which siderotic granules could be detected (by ordinary light microscopy) Both of the groups of workers cited above demonstrated therefore that the presence of sidero

tic granules in erythroblasts was a normal phenomenon, they pointed *out, too, that the* proportion of erythroblasts containing the granules and their number and size were increased in conditions where there was evidence of defective erythropoiesis or haemoglobin synthesis Douglas and Dacie (14), *moreover, illustrated the* occurrence of large siderotic granules surrounding the nucleus in the form of a collar in a patient considered (at the time of writing) to have an "acquired defect of haemoglobin synthesis" Details of this patient (who undoubtedly had sideroblastic anaemia) were published 6 years later (10) (Case 1)

The first definitive description of a chronic refractory anaemia in which sideroblasts in the marrow were the outstanding feature was provided by Bjorkman (4) The illness in three patients in this series was benign and long continued but the fourth patient died of myeloblastic leukaemia Since this time many more cases of "sideroblastic" anaemia have been described and it is now realized that these anaemias occur far more frequently than was it first thought likely The pathogenesis of the sideroblastic anaemias — they comprise a group — is still poorly understood and it is the purpose of the present paper to review briefly present knowledge in this field, with particular reference to the role of the spleen in controlling the number of siderocytes in the peripheral blood, types of sideroblasts the classification of the sideroblastic anaemias the role of deficiencies of pyridoxine and other vitamins in the production of sideroblasts and the relationship between sideroblastic anaemias and leukaemia

Siderocytosis and Splenectomy

As already pointed out, siderocytes are not found in appreciable numbers in the peripheral blood in health although a small percentage (0 to 3% of marrow erythrocytes) can be demonstrated amongst marrow reticulocytes (14) on the other hand, some *siderocytes seem always to* be present in the peripheral blood after splenectomy, unless there is iron deficiency The iron demonstrable in normal normoblasts and reticulocytes is iron taken into developing cells in excess of that which is immediately required for haemoglobin synthesis This excess iron is in all probability utilized during the completion of haemoglobin synthesis which occurs during the final ripening of the reticulocyte There is evidence to suggest that reticulocytes soon after *leaving the marrow* become sequestered in the spleen (in health as well as in disease) for a matter of hours or even a day or so (1) (24) and it seems reasonable to suppose that the iron demonstrable in these reticulocytes is utilized at this time for the continuing synthesis of haem In consequence when the reticulocyte finally escapes from the spleen into the peripheral circulation too little iron is present for it to be demonstrable as granules visible by *light microscopy After splenectomy*, it seems likely that the maturation of

Table I Types of Sideroblast

1	*Normal normoblasts*	small granules difficult to see
2	*Normoblasts (or megaloblasts)*	with more and larger granules easily visible as in haemolytic anaemia, megaloblastic anaemia, thalassaemia, haemochromatosis, transfusional siderosis
3	*Pathological sideroblasts*	many granules often very large "ring" sideroblasts characteristic, as in 1ry and 2ry "sideroblastic" anaemias

reticulocytes takes place in the circulating blood to a greater extent. This means that some peripheral blood reticulocytes will be siderocytes, which in fact is what is found.

In certain pathological states (see below), where more iron accumulates in the developing cells than can be utilized for haemoglobin synthesis, the erythroblasts and reticulocytes may contain far more and larger siderotic granules than normal. With the spleen *in situ* it seems that this excess of iron can be removed, probably usually at the reticulocyte stage, by a mechanism referred to by Crosby as "culling". Crosby (8) in cross transfusion experiments showed, too, that the spleen was *capable of removing large siderotic* granules from adult erythrocytes. Furthermore, histological sections of the spleen from patients with sideroblastic anaemia invariably show an excess ac*cumulation of fine iron granules in* littoral cells lining the sinusoids. On the other hand, if patients whose erythroblasts contain more iron than can be utilized for haemoglobin synthesis *undergo* splenectomy, this iron remains in reticulocytes, and later in adult erythrocytes until the end of the cells' life span because there seems to be no mechanism for its removal except the spleen. Hence the very high siderocyte counts which may be found *after splenectomy* in patients suffering from disorders of haem synthesis.

Three Types of Sideroblast

The term sideroblast was first introduced by Kaplan, Zuelzer and Mouriquand (2[illegible]) to describe an erythroblast with iron containing granules visible in its cytoplasm. Subsequently it has been used to describe the type of sideroblast seen in sideroblastic anaemia. This is confusing but unfor*tunately there is no easy solution to* this problem of nomenclature. The present authors recognize three types of sideroblast (Table I). The first is the sideroblast seen in normal mar*rows: the iron containing granules* are few in number and difficult to see; they are very difficult to photograph and are in any case only visible *in a* proportion of erythroblasts.

The second type of sideroblast is found in conditions in which the per

centage saturation of transferrin is increased They are therefore found in conditions in which the body stores of iron are increased (haemochromatosis and transfusional siderosis) or in which there is excessive haemolysis or in which there is dyshaemopoiesis (as in megaloblastic anaemia or thalassaemia) The granules in such cases are larger than normal and may be more numerous, they are diffusely scattered in the cells' cytoplasm and seldom if ever form a conspicuous ring or collar around the nucleus Almost all cells are affected

The third type of sideroblast is found in the sideroblastic anaemias The siderotic granules are larger than normal and many are present, they may be diffusely scattered throughout the cytoplasm but in some cells at least they may form a conspicuous ring or collar around the nucleus These are "ring or "ring type" sideroblasts The cause of the ring arrangement is not clear In some cases at least the iron in this position is located in perinuclear mitochondria (2) To some extent, too the granules may be crowded around the nucleus because of poor development of the cells cytoplasm Excess iron stores and a high serum iron concentration are frequent findings in these cases, too, and the effect of this is exacerbated by impaired haem synthesis leading to defective iron utilization In some patients, however ring-sideroblasts may be seen when the percentage saturation of transferrin is normal or only slightly raised

The Sideroblastic Anaemias

We should like to define a sideroblastic anaemia as an anaemia in which there is clear evidence for abnormal sideroblastic development and in which some at least of the sideroblasts are of the ring type The presence of the abnormal sideroblasts is associated with hypochromia of the peripheral blood erythrocytes but, rather characteristically, normochromic cells are present also, in varying proportion, the result is a well marked dimorphic picture Occasionally the hypochromic cells are few and far between

Table II Types of Sideroblastic Anaemia

Hereditary		Sex linked hypochromic anaemia
Acquired		
	Primary	Refractory sideroblastic anaemia
	Secondary	Myeloproliferative diseases
		Malabsorption syndrome rheumatoid arthritis carcinoma etc
		Drugs INH cycloserine lead. alcohol

Sideroblastic anaemia is *not* a single disorder, there are in fact both inherited and apparently acquired types The acquired types can be separated into two groups, namely, primary (or idiopathic) cases and cases secondary to or associated with a variety of underlying diseases (Table II) Sideroblastic anaemia too, may result from the use of certain drugs, for example, patients on anti tuberculosis drugs or with chronic alcoholism may show this picture and we have recently found sideroblastic change associated with haemolytic anaemia in five patients with partial gastrectomy who

Table III Hereditary Sideroblastic Anaemia

Age of onset 6—30 years	*Sex* usually male
Family history characteristically positive	
Anaemia hypochromic often dimorphic	
MCHC 22—28 %	*MCV* 74—84 cu μ
Reticulocytes 1—4 %	*Siderocytes* many after splenectomy
Hb F absent or trace	*Hb A_2* reduced
^{51}Cr $T_{1/2}$ slightly decreased	*Serum Fe* increased
	TIBC saturation raised

Partial response to pyridoxine and/or folic acid not infrequent

were taking large doses of phenacetin or Panadol (p acetamidophenol) In respect of the multiplicity of causes the situation may be looked upon as analogous to the megaloblastic anaemias where interference with vitamin B_{12} or folic acid metabolism can be brought about in a number of ways The final result — megaloblastic change in the marrow — is however identical whatever the cause and the same seems to be generally true of the morphological changes of sideroblastic anaemias

Hereditary Sideroblastic Anaemia

This is a rare disorder but as its existence becomes better known no doubt more cases will be recognised It seems first to have been described although not at the time recognized as a sideroblastic anaemia by Cooley (6) and Rundles and Falls (34) The disorder presents as a chronic hypochromic anaemia affecting males particularly The anaemia may be of severe grade and necessitate blood transfusion

The diagnosis has generally been made in adolescence females are less severely affected and here the diagnosis is usually made in the course of family studies Inheritance typically follows a sex linked apparently partially recessive pattern (34) (27) The prognosis (for males) is not good and deaths have particularly resulted from thromboembolism consequent on splenectomy

The patient s illness is often misdiagnosed as an iron deficiency anaemia In reality although the blood picture in respect of its hypochromia may superficially point to this diagnosis there is no deficiency of iron On the contrary more often than not the marrow and liver and other organs usually contain a great excess as the result of previous futile iron salt therapy The main haematological findings are summarized in Table III

Hereditary sideroblastic anaemia superficially resembles thalassaemia but it can be distinguished from thalassaemia by its different mode of inheritance haematologically although the blood pictures have many points of similarity sideroblastic anaemia is characterized by subnormal amounts of Hb A (32) and by relatively low levels of Hb F There is also the important difference that in thalassaemia

ring sideroblasts are absent or present in only very small numbers. From the therapeutic point of view there is a chance of a response to pyridoxine therapy (see later), which is not the case in thalassaemia.

Acquired Sideroblastic Anaemias

Our personal experience as to the relative incidence of primary (idiopathic) and secondary acquired cases is summarized in Table IV. One third or more of the patients had the primary disease. This relatively large group of patients represents all the patients diagnosed as having sideroblastic anaemia who have been studied at Hammersmith Hospital over the last 16 years. Clinical and haematological details of the first seven primary cases have already been published (10).

Although it is true that many of our patients were referred to us because of our known interest in the disorder, it seems clear that primary acquired sideroblastic anaemia is not a rare disease, in fact it seems to be the commonest cause of refractory dyshaemopoietic anaemia of obscure origin occurring in adults, if leukaemia and aplastic anaemia are excluded. But cases will not be recognized at all frequently unless bone marrow films are stained for iron as a routine.

Table IV. Primary and Secondary Acquired Sideroblastic Anaemia

	No. of cases
1. *Primary acquired sideroblastic anaemia*	
[1] Refractory anaemia in older patients of either sex	23
Presentation other than anaemia (e.g. fracture, pruritus, pyrexia, haematuria)	6
Total	29
2. *Secondary acquired sideroblastic anaemia*	
Rheumatoid arthritis	6
Polyarteritis nodosa	1
Leukaemia	3
Polycythaemia vera, thrombocythaemia or myelofibrosis	6
Myeloma	[illegible]
Carcinoma	4
Haemolytic anaemia (hereditary spherocytosis, elliptocytosis)	3
P.A., malabsorption syndrome and partial gastrectomy	[illegible]
Myxoedema	[illegible]
Drugs	
Isoniazid (INH) therapy	3
Alcoholism — dietary deficiency	[illegible]
[2] Phenacetin, Panadol (*p*-acetamidophenol)	5
[3] Lead	[illegible]
Imuran (azathioprine)	1
Total	[illegible]

[1] One patient developed sideroblastic anaemia eight years after being treated for six months with Neomercazole for thyrotoxicosis.

[2] These patients had partial gastrectomy or blind loop syndrome. There was also an associated mild haemolytic anaemia.

[3] One patient had severe acute lead poisoning, the other was exposed to lead for sixteen years and developed overt sideroblastic anaemia twenty years after exposure.

Primary ("Idiopathic") Acquired Sideroblastic Anaemia

The disorder most commonly affects middle aged or elderly subjects. The age range in our series is 37—86 years, the mean age on diagnosis being 70 years. Both sexes have been affected, 18 females to 11 males.

The patients usually have presented with symptoms attributable to anaemia extending over a period of months or even years. Often they have received previous treatment with vitamin B_{12} or iron without effect. Physi

cal examination is usually unrewarding However, the spleen may be just palpable

The peripheral blood picture is characteristic and may permit a tentative diagnosis to be made The abnormality which if appreciated suggests sideroblastic anaemia is the occurrence of a minority of hypochromic erythrocytes amid many more or less normal looking cells The proportion of hypochromic cells varies from case to case they are never absent but in our experience they do not dominate the picture as they do in hereditary cases Anisocytosis poikilocytosis and macrocytosis are present to a variable degree Erythroblasts are seldom seen and the leucocyte and platelet counts are usually normal Siderocytes are typically absent or present in only very small numbers unless the spleen has been removed then many extremely hypochromic cells containing large siderotic granules will be present

The bone marrow is characteristically hypercellular with erythropoiesis dominant Nearly every erythroblast contains an excess of siderotic granules and many of them will be ring forms The marrow particles contain as a rule a great excess of iron Typically this is mainly in the form of many small fine granules of approximately the size of the siderotic granules seen in developing erythroblasts It appears probable in fact that the extra erythroblast marrow iron is in large part derived from siderotic granules originating in erythroblasts or reticulocytes which have failed to mature or from similar granules extruded from developing cells in the course of successful maturation

Megaloblastic change (overlay) is not uncommon in some cases this may be marked and easy to diagnose in others the changes are minimal This is due to deficiency of folic acid not vitamin B_{12} (see below)

Deficiency of Folic Acid and Pyridoxine

Deficiency of folic acid and pyridoxine is relatively frequent in sideroblastic anaemia this applies probably to both the hereditary and acquired types Amongst our series of primary cases there were 80 % in which the serum folate concentration was subnormal and 75 % of those who were tested gave positive FIGLU tests Positive FIGLU tests and excessive excretion of urocanic acid were however noted in some patients with no biochemical or haematological evidence of folate deficiency Approximately 50 % excreted xanthurenic acid after a tryptophane load indicating deficiency of pyridoxine or derangement in its metabolism The cause of the folic acid (folate) deficiency seems likely to be an increased requirement due to greatly increased although largely ineffective erythropoiesis in addition a defect in pyridoxine metabolism may interfere with reactions dependent on folate The cause of the apparent pyridoxine deficiency is probably even more complex Increased demand may be a factor but actual interference

with pyridoxine metabolism is likely to be important in many cases (see below)

Response to Folic Acid, Pyridoxine and Crude Liver Extract

It is now widely known that some patients suffering from sideroblastic anaemia respond to folic acid therapy. This was so in approximately one third of our cases of either the primary acquired or hereditary type. The response was, however, generally small and in no case was a normal haemoglobin level achieved. Responses when present were, however, associated with reversal of the marrow picture from megaloblastic to normoblastic.

Some of our patients with hereditary or primary acquired sideroblastic anaemia have shown further responses to pyridoxine, sometimes to small doses, e.g. 0.5 to 1.0 mg per day; other patients have seemed to need relatively enormous doses. The rise in haemoglobin is often limited and in only a few cases has a normal level been reached and even in these patients minor abnormalities have persisted in the peripheral blood and bone marrow. Two of our patients who were receiving folic acid and pyridoxine, showed some response to crude liver extracts [presumed to contain the haemopoietic factor of Horrigan, Whittington, Weisman and Harris (23)].

Secondary Acquired Sideroblastic Anaemia

In Table IV is shown the wide range of disorders in which ring sideroblasts in the marrow have been encountered. In some of these patients the role of the sideroblastic change in relation to the patients' anaemia seems to have been a minor one, in others it has probably been a major cause of their anaemia.

The cause of the sideroblastic change in these cases is obscure. Evidence is already available that lead can interfere with haem synthesis at various points. Presumably the anti-tuberculosis drugs act by interfering with pyridoxine metabolism, but at what stage derangement of pyridoxine metabolism interferes with the synthesis of haemoglobin is uncertain (see below). The different anti-tuberculosis drugs differ markedly in their "sideroblastic anaemia producing" effect. Drugs like cycloserine and pyrazinamide appear to produce sideroblastic anaemia far more readily in man and experimental animals than does INH. However recent studies indicate that abnormalities qualitatively similar to those seen in sideroblastic anaemia but of lesser intensity occur in the bone marrows of a very large proportion of patients receiving INH (33).

The association of leukaemia with sideroblastic change in the marrow is of special interest and has received a good deal of attention (11). In our experience ring sideroblasts are not commonly seen in leukaemia; occasionally, however, a few are present. They seem to be relatively more frequent in myelosclerosis. Exceptionally, in our experience many are present in both leukaemia and myelosclerosis and in

rare instances the distinction between chronic Di Guglielmo's disease (with sideroblastic change) and primary acquired sideroblastic anaemia may be difficult. But we believe that the majority of patients with acquired sideroblastic anaemia certainly do not have leukaemia. The transformation of the former to the latter may sometimes occur but we do not believe that this often happens. In our opinion there seems no reason why sideroblastic change to a minor or major degree should not occur from time to time in leukaemia as one aspect of the alteration in marrow cell metabolism consequent on leukaemic change.

Pyridoxine Responsive Anaemia

There is now a large literature on the anaemias responsive to pyridoxine and the subject has recently been fully reviewed (22). In the present authors' view these anaemias do not need to be placed in a separate category; they are sideroblastic anaemias of hereditary or acquired types. Although many of these patients respond to treatment with pyridoxine there may be no clinical or laboratory evidence of pyridoxine deficiency.

Some patients have however been recorded in whom the biochemical tests are indicative of pyridoxine deficiency and who respond almost completely to relatively small doses of pyridoxine e.g. 1 mg daily by mouth (29), 5 mg daily by mouth (15). In these patients the condition rapidly relapses if treatment is stopped. The patient studied by MacGibbon and Mollin (29) took a very poor diet but as some hypochromia and a few ring sideroblasts persisted even after very large doses of pyridoxine it is unlikely that the metabolic basis for the disorder, even in these patients, is uncomplicated pyridoxine deficiency. This is supported by the fact that sideroblastic change is rarely seen in patients with other evidence of nutritional deficiency or in patients with the intestinal malabsorption syndrome. This may be because such patients are usually iron deficient (12). But this is unlikely to be the whole explanation, for ring sideroblasts rarely develop when the iron deficiency is corrected.

On the other hand, in some secondary sideroblastic anaemias it seems likely that pyridoxine deficiency alone or probably more effectively when associated with folic acid deficiency can produce sideroblastic change. In some of these patients hypochromia and ring sideroblasts disappear when the patients are treated with pyridoxine and folic acid. In other cases these abnormalities disappear with remission of the associated disease.

It seems likely therefore that in certain secondary cases pyridoxine deficiency can by itself, or when associated with folic acid deficiency, result in a sideroblastic anaemia which is apparently cured when pyridoxine is given as treatment. The results in cases which have followed the use of anti-tuberculosis drugs which are known to act as pyridoxine antago

nists support this view (26) (31) (28) (36)

It is quite clear that pyridoxine deficiency is *not* the cause of primary acquired sideroblastic anaemia Instead, an increased requirement for pyridoxine (and folic acid) often appears to be superimposed on an acquired disorder of erythropoiesis — which itself, too, produces ring sideroblasts Response to pyridoxine and/or folic acid in these cases depends upon the degree and severity of the underlying abnormality and the extent of the overlying deficiency state Where the response only follows the giving of very large doses of pyridoxine, replenishment of a conditioned deficiency can hardly be the explanation In these cases it seems more likely that pyridoxine is overcoming partially, by mass action perhaps a defect in a metabolic process in which a derivative of pyridoxine acts as a co factor

The Nature of Sideroblastic Anaemia

The disturbance leading to ring sideroblast formation and to sideroblastic anaemia is uncertain It has been suggested that the primary defect is interference with the synthesis of haem The synthesis of protoporphyrinogen from glycine and succinate via amino laevulic acid porphobilinogen uroporphyrinogen and coproporphyrinogen and its final linkage with iron to form haem is mediated through a whole system of enzymes and interference or deficiency of some of these enzymes may be the basis of a haem synthesis defect in sideroblastic anaemia Pyridoxine is a key substance in this chain of syntheses, for the combination of glycine and succinyl Co-A is brought about by a system requiring pyridoxal or pyridoxal 5 phosphate Pyridoxal 5 phosphate is also required for the mobilization of iron from mitochondria (7) We have already pointed out that a condition closely resembling naturally occurring sideroblastic anaemia can be produced in man and in experimental animals by drugs that act as pyridoxine antagonists, while patients with naturally occurring sideroblastic anaemia sometimes show evidence of abnormal pyridoxine metabolism

It should be added that Vavra and Poff (35) could find by in vitro studies no direct evidence of a defect in synthesis between amino laevulic acid and haem in eight patients with sideroblastic anaemia the defect, however, may occur earlier Thus lead which is a well known cause of sideroblastic anaemia, affects haem synthesis prior to amino laevulic acid formation it also affects, however, the incorporation of iron into protoporphyrin and probably acts on other stages of porphyrin synthesis, too (16)

A good deal more needs to be known before it is justifiable to come to any firm conclusions as to the pathogenesis of the sideroblastic anaemias Pyridoxine antagonists act on many enzyme systems and could therefore affect haemoglobin synthesis less directly than we have suggested and there is some evidence which suggests that globin synthesis is ineffec

tive in some types of sideroblastic anaemia (21)

The role of excess intracellular iron itself is particularly interesting but still needs clarification It is possible that excess iron present in erythroblasts due to the combined effects of high availability defective utilization and an increased rate of uptake of iron by the erythrocyte (5) acts as an impediment to haem synthesis The demonstration of the enormous accumulation of iron in mitochondria in sideroblastic anaemia suggests that mitochondrial activity may be impaired and experimentally Harriss MacGibbon and Mollin (20) showed that drug induced sideroblastic anaemia in mice and guinea pigs tended to be more severe in animals given an iron supplement The argument that excess iron cannot be of much importance because haemochromatosis is *not* accompanied by anaemia is not sound for intra erythroblast iron is not greatly increased in haemochromatosis and ring sideroblasts are not seen However it has to be admitted that Bishop and Hathaway (3) failed to demonstrate that iron inhibited haem synthesis in an in vitro system but the results in the experimental system they used may not be entirely applicable to the unusual conditions found in sideroblastic anaemia

Summary

The circumstances in which siderocytes and sideroblasts occur in human peripheral blood and bone marrow are briefly reviewed The different types of sideroblastic anaemia are described and their pathogeneses discussed

Acknowledgements

Dr D L Mollin acknowledges receipt of grants from the Medical Research Council.

References

1 Berendes M Blood *14* 558 1959
2 Bessis M C & Jensen W N Brit J Haemat *11* 49 1965
3 Bishop P C & Hathaway T R Clin Res 8 207 1960
4 Bjorkman S E Blood *11* 250 1956
5 Brozovic B & Mollin D L (To be published 1966)
6 Cooley T B Amer J med Sci 209 561 1945
7 Cooper R G Webster L T & Harris J W J clin Invest 42 926 1963
8 Crosby W H Blood *12* 165 1957
9 *Dacie J V & Doniach I J Path Bact* 59 684 1947
10 Dacie J V Smith M D White J C & Mollin D L Brit J Haemat, 5 56 1959
11 Dameshek W Brit. J Haemat. *11* 52 1965
12 Dawson, A M Holdsworth C D & Pitcher C S Gut 5 504 1964
13 Doniach I., Gruneberg H & Pearson, J E G J Path Bact 55 23 1943
14 Douglas A S & Dacie J V J clin *Path 6 307* 1953
15 Gehrmann G Brit J Haemat. *11* 86 1965
16 Goldberg A Brit. J Haemat *11* 114 1965
17 *Gruneberg H Nature (Lond) 148 114* 1941
18. Gruneberg H Nature (Lond) *148* 469 1941
19 Gruneberg H J Genet. 44 246 1942
20 Harriss E B, *MacGibbon B H* & Mollin, D L. Brit J *Haemat 11* 99 1965
21 Heller P., Stone, J V Apple, D & Coleman, R. D Blood 25 635 1965

22 Horrigan D L & Harris J W Advanc intern Med 12 103 1964

23 Horrigan D L Whittington R M Weisman R & Harris J W Amer J Med 22 99 1957

24 Jandl J H J Lab clin Med 55 663 1960

25 Kaplan L Zuelzer, W W & Mouriquand, C Blood 9 203 1954

26 Kohn R Heilmeyer L & Clotten R Dtsch Med Wschr 87 1765 1962

27 Losowsky M S & Hall R Brit J Haemat 11 70 1965

28 McCurdy P R Clin Res 11 59 1963

29 MacGibbon B H & Mollin D L Brit J Haemat 11 59 1965

30 Pappenheimer, A M Thompson W P Parker D D & Smith K E Quart. J Med 14 75 1945

31 Redleaf P D Dis Chest 42 [illegible] 196[illegible]

32 Reed J L and Mollin D L (To be published 1966)

33 Roberts P D, Hoffbrand A V & Mollin D L Brit med J (To be published, 1966)

34 Rundles R W & Falls H F Amer J med Sci 211 641 1946

35 Vavra D D & Poff S A J clin Invest. 44 1106 1965

36 Verwilghen R Reybrouck G Callens L & Cosemans J Brit J Haemat 11 92 1965

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Hypochromic Anaemia without Iron Deficiency

By M. C. Verloop, P. W. Heileman and K. Punt

In our contribution to this festive publication commemorating *Jan Waldenström's* 60th anniversary, we should like to discuss some unusual causes of hypochromic anaemia. Sideropenic hypochromic anaemia has been investigated by many authors, including *Waldenström*, who as early as 1937 pointed out cutaneous and mucosal changes (24) and described sideropenic dysphagia (25).

Occasionally, however, one encounters patients with hypochromic anaemia who do not suffer from sideropenia but in fact often show the opposite, haemosiderosis. We intend to present a brief description of some forms of this hypochromic "hypersideraemic" anaemia.

Introduction

The blood picture in patients with non-sideropenic hypochromic anaemia is usually microcytic. The blood smear is also characterized in many cases by large, intensively hypochromic cells known as target cells. Partly due to the presence of these "flat" target cells, the mean red cell thickness is often diminished, while the resistance of the red cells to hypotonic saline solutions in decreasing concentrations is increased.

Differential diagnosis of hypochromic hypersideraemic anaemias

When in North West Europe one encounters a hypochromic anaemia not based on iron deficiency, three disease pictures must be borne in mind as primary alternatives, namely thalassaemia minor, α thalassaemia and hereditary sideroachrestic anaemia.

In thalassaemia, synthesis of normal adult Hb A is diminished because there is a genetically determined disturbance in the production of either the alpha or the beta chains of the globin, giving rise to alpha thalassaemia or beta thalassaemia respectively.

Thalassaemia minor (β thalassaemia)

Thalassaemia minor — the heterozygous form of beta thalassaemia — can be found in all countries of W. Europe. It is also observed in patients whose ancestors do not seem to come from

Table I *Thalassaemia minor* (*β-thalassaemia*)

Patients	Sex and Age	Hb g/100 ml	Erythrocytes mill./cu mm	M C H[1] μμg	M C V[2] μ3	Serum iron Fasting μg/100 ml	Serum iron Saturation %
Normal range				28—32	80—100	80—180	20—60
Fam 1	F 18	10.1	4.3	24	60	138	3[illegible]
Fam 2	M 34	10.6	5.4	20	63	167	51
3	M 19	10.9	4.8	29	83	142	33
4	F 61	9.8	4.5	22	80	136	44
5	F 5	9.8	4.3	23	84	151	56

Patients	Bone marrow: Ratio Red White	Bone marrow: R E S iron	Bone marrow: Sideroblasts	Bone marrow: Ringed sideroblasts %	Haemoglobin: Alkaline resistant Hb %	Haemoglobin: A1 %	Haemoglobin: A2 %	Haemoglobin: H %
Normal range	1 (2.5—5)	± or +	±	0	max 3	max 98	max 3	0
Fam 1	1 2	++	+	0	0.9	94.3	4.8	0
Fam 2	1 1	++	+	0	2.2	93.1	4.7	0
3	2 1	+	+	0	1.4	93.3	4.7	0
4	1 1	++	+	0	9.3	98.5	5.7	0
5					3.8	91.3	4.9	0

[1] M C H = Mean Corpuscular Haemoglobin
[2] M C V = Mean Corpuscular Volume

countries on the Mediterranean sea (5, 18). The disturbance in haemoglobin formation results in a usually mild to moderately severe hypochromic anaemia, which is often microcytic. Table I presents data on some Dutch families studied by us. The serum iron concentration is normal or slightly increased with usually a normal iron saturation percentage of transferrin. The bone marrow shows haemosiderosis, and a sideroblast count which is slightly above normal. So called ringed sideroblasts — i.e. erythroblasts in which a ring of thick iron granules closely surrounds the nucleus — are sporadically seen, if at all. The condition is diagnosed on the basis of haemoglobin analysis. The haemoglobin A_2 concentration is as a rule increased and sometimes the haemoglobin F concentration too.

Alpha-thalassaemia (haemoglobin H disease)

The second possibility to be taken into account in the case of "iron saturated" hypochromic anaemia is alpha thalassaemia.

In alpha thalassaemia the production of alpha chains of the globin is retarded, as a result of which the normal haemoglobin synthesis is disturbed and an excess of free beta chains

Table II α *Thalassaemia (Haemoglobin H disease)*

Patient	Sex and Age	Hb g./100 ml	Erythrocytes mill./cu mm.	M C H[1] μμg	M C V[2] μ3	Serum iron Fasting μg./100 ml.	Serum iron Saturation %
Normal range				28—32	80—100	80—180	20—60
1	M 45	10 5	5 9	18	67	98	37

	Bone Marrow: Ratio Red White	Bone Marrow: R E S iron	Bone Marrow: Sideroblasts	Bone Marrow: Ringed sideroblasts %	Haemoglobin: Alkaline resistant Hb %	Haemoglobin: A_2 %	Haemoglobin: A %	Haemoglobin: H %	Erythrocytes with B C B[3] incl. bod %
Normal range	1 2 5 5)	± or +	±	0	max 3	max. 98	max 3	0	0
1	2 1	++	+	0	0 9	98 4	0 7	2—4	73

[1] M C H = Mean Corpuscular *Haemoglobin*
[2] M C V = Mean Corpuscular Volume
[3] B C B = Brilliant Cresyl Blue

can occur These chains can polymerize to become tetramers and combine with haem to form a new haemoglobin known as haemoglobin H (β_4) Shortly after birth when haemoglobin synthesis is still largely confined to haemoglobin F an excess of gamma chains can lead in these cases to the formation of Bart s haemoglobin whose globin part consists of a tetramer of gamma chains The disturbed production of the alpha chains can give rise *to a decrease in haemoglobin A* The alpha chain deficiency also leads to a *more* or less *severe* hypochromic anaemia Variations in the manifestations *of alpha thalassaemia have been* described in *Weatherall* s monograph (26)

In alpha thalassaemia the amount of haemoglobin H and/or Bart s haemoglobin is usually too small to be readily demonstrable but demonstration is sometimes possible in these cases by means of a sensitive starch gel electrophoresis technique (15) Moreover a suspicion of alpha thalassaemia warrants a search for typical red cell inclusions caused by denaturation of labile haemoglobin H These inclusions can be detected after incubating the erythrocytes with brilliant cresyl blue (19)

Table II presents a number of data on a patient of Dutch extraction with alpha thalassaemia or haemoglobin H disease The hypochromic anaemia in alpha thalassaemia like that in beta thalassaemia is often microcytic *Again the serum iron concentration* is normal or slightly increased with usually *a normal serum iron* saturation percentage The erythropoietic system in the *bone marrow is often* hyperplastic, with haemosiderosis in the reticulo endothelial system *As in the*

Table I *Thalassaemia minor* (β-*thalassaemia*)

Patients	Sex and Age	Hb g/100 ml	Erythrocytes mill./cu mm	M C H[1] μμg	M C V μ3	Serum iron	
						Fasting μg/100 ml	Saturation %
Normal range				28—32	80—100	80—180	20—60
Fam 1	F 18	10 1	4 3	24	60	138	37
Fam 2	M 34	10 6	5 4	20	63	167	51
3	M 19	10 9	4 8	29	83	142	33
4	F 61	9 8	4 5	22	80	136	44
5	F 5	9 8	4 3	23	84	151	36

Patients	*Bone marrow*				*Haemoglobin*			
	Ratio Red White	R E S iron	Sideroblasts	Ringed sideroblasts %	Alkaline resistant Hb %	A_1 %	A_2 %	H %
Normal range	1 (2 5—5)	±or+	±	0	max 3	max 98	max 3	0
Fam 1	1 2	++	+	0	0 9	94.3	4 8	0
Fam 2	1 1	++	+	0	2 2	93 1	4 7	0
3	2 1	+	+	0	1 4	93.3	4 7	0
4	1 1	++	+	0	9 3	98 5	5 7	0
5					3 8	91.3	4 9	0

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countries on the Mediterranean sea (5, 18) The disturbance in haemoglobin formation results in a usually mild to moderately severe hypochromic anaemia, which is often microcytic Table I presents data on some Dutch families studied by us The serum iron concentration is normal or slightly increased with usually a normal iron saturation percentage of transferrin The bone marrow shows haemosiderosis, and a sideroblast count which is slightly above normal So called ringed sideroblasts — i e erythroblasts in which a ring of thick iron granules closely surrounds the nucleus — are sporadically seen if at all The condition is diagnosed on the basis of haemoglobin analysis The haemoglobin A_2 concentration is as a rule increased and sometimes the haemoglobin F concentration too

Alpha thalassaemia (haemoglobin H disease)

The second possibility to be taken into account in the case of "iron saturated" hypochromic anaemia is alpha thalassaemia

In alpha thalassaemia the production of alpha chains of the globin is retarded, as a result of which the normal haemoglobin synthesis is disturbed and an excess of free beta chains

Table IV "*Thalassaemia like Syndrome*"

Patients	Sex and Age	Hb g./100 ml	Erythrocytes mill./cu mm	M C H[1] μμg	M C V μ3	Serum iron: Fasting μg./100 ml	Serum iron: Saturation %
Normal range				28—32	80—100	80—160	20—60
Fam 1	M 70	10 9	5 3	20	72	186	56
Fam 2	M 30	13 4	5 2	22	80	138	43
Fam 3	M 66	13	5 6	23	73	131	46

	Bone marrow: Ratio Red White	Bone marrow: R E S iron	Bone marrow: Sideroblasts	Bone marrow: Ringed sideroblasts %	Haemoglobin: Alkaline resistant Hb %	Haemoglobin: A1 %	Haemoglobin: A2 %	Haemoglobin: H %	Erythrocytes with B C B[3] incl. bod %
Normal range	1 (2 5—5)	± or +	±	0	max. 3	max 98	max 3	0	0
Fam 1	1 1	++	+	0	1 3	96 2	2.5	0	absent
Fam 2	1 1	++	+	0	1 8	95 8	2 4	0	absent
Fam 3					1.3	96 1	2 6	0	absent

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and iron saturation of transferrin repeatedly attains 100 %. In the bone marrow the hyperactive erythropoietic system is usually normoblastic. There is iron storage and the RES of the bone marrow always shows pronounced haemosiderosis. Bone marrow smears stained for iron moreover show typical sideroblasts, the so called ringed sideroblasts, in which thick granules of iron are found in the mitochondria immediately surrounding the nucleus (4).

Thalassaemia like syndrome"

Sometimes in cases of hypochromic "hypersideraemic" anaemia no signs of the three just described diseases can be detected.

We found an autosomal hereditary hypochromic microcytic anaemia without any sign of alpha or beta thalassaemia in three consecutive generations of the same Dutch family. The anaemia was also found in a female member; these facts ruled out a hereditary sex linked sideroachrestic anaemia. This syndrome is to be discussed in detail elsewhere (7).

Table IV presents data on some members of this family. This hypochromic anaemia again entails a normal to slightly increased serum iron concentration and bone marrow haemosiderosis. Ringed sideroblasts are absent. An exhaustive electrophoretic study up till now failed to disclose an abnormal haemoglobin and there was

Table III *Hereditary Sideroachrestic Anaemia*

Patients		Sex and Age	Hb g/100 ml	Erythrocytes mill./cu mm	M C H[1] μμg	M C V[2] μ3
Normal range					28—32	80—100
Fam	1	M 39	10.6	5.3	20	75
	2	M 38	9.0	4.4	20	70
	3	M 36	12.0	5.6	21	67
	4	M 28	9.9	4.5	22	71
Fam	5	M 54	7.5	5.0	15	52
	6	M 57	11.4	5.3	21	74

		Serum iron		Bone Marrow			
		Fasting μg/100 ml	Saturation %	Ratio Red : White	R E S iron	Sideroblasts	Ringed sideroblasts %
Normal range		80—180	20—60	1 : (2.5—5)	± or +	±	0
Fam	1	236	95	1 : 1	+++	+++	48
	2	174	49				
	3	124	33				
	4	212	71				
Fam	5	262	80	1½ : 1	+++	++	38
	6	141	36	1 : 1	+++	++	20

[1] M C H = Mean Corpuscular Haemoglobin
[2] M C V = Mean Corpuscular Volume

lassaemia minor the sideroblast count is increased, but so called ringed sideroblasts are absent

This patient is the first of Dutch extraction to be described with this condition A few other authors (3 12 22, 29) have likewise described alpha thalassaemia in patients of North West Europe in origin

Hereditary sideroachrestic anaemia

The third alternative of hypochromic hypersideraemic anaemia is that which occurs as a familial condition in males The condition is probably subject to X chromosomal transmission and is known as hereditary sideroachrestic anaemia It was first described by *Cooley* (7) and subsequently by many other investigators (13 17 20 27) No abnormal haemoglobin is found in these patients and there are no changes in the amount of haemoglobin A2 or haemoglobin F

Table III presents a number of data on a few patients with this condition whom we examined As in the case of thalassaemia minor and in haemoglobin H disease there is a hypochromic often microcytic anaemia which can range from mild to severe The serum iron concentration is often increased

without any iron deficiency in the organism (11 16)

To summarize it is of importance to consider the above described diseases as possibilities in the case of hypochromic anaemia refractory to iron medication and to bear in mind that protracted iron medication may harm these patients who may well show a favourable response to some other form of treatment

References

1 Bannerman R M Thalassaemia Grune and Stratton New York 1961
2 Beaven G H Stevens B L Ellis M J White J C Bernstock L Masters P and Stapleton T Brit J Haemat 10 1 1964
3 Bergren W R and Sturgeon P H Proc of Int Soc of Haemat vol II part I 488 1960
4 Bessis M and Breton Gorius J Blood 14 423 1959
5 Callender S T Mallett B J and Lehmann H Brit J Haemat. 7 1 1961
6 Ceppellini R Acta Genet Med (Roma) 8 suppl 2 (Symposium Geneticae Haematologicae) 47 1959
7 Cooley Th B Am J Med Science 209 561 1945
8 Gehrmann G Folia Haemat (Frankfurt) N F 2 275 1958
9 Gouttas A Fessas Ph Tsevrenis H and Xefteri M I Sang 26 911 1955
10 Gouttas A Haemoglobin disorders in the Mediterranean region Ciba Symposium 17 3 1964
11 Haurani F S Young K and Tocantins L M Blood 22 73 1963
12 Hedenberg F, Muller Eberhard H., Sjolin S and Wranne L Acta Paed 47 652 1958
13 Heilmeyer L., Emmrich J Hennemann M H Schubothe H., Keiderling W., Lee M Bilger R and Bernauer W Schweiz Med W'schrift 87 1237 1957
14 Heilmeyer L Keller W Vivell O Keiderling W., Betke K Wohler F and Schultze H E Deutsche Med W'schr 86 1745 1961
15 Helleman P W and Gartner H Nature 201 833 1964
16 Lee S L Rosner F., Rivero I Feldman F and Harwitz A New Engl J Med 272 761 1965
17 Losowsky M S and Hall R Brit J Haemat 11 70 1965
18 Marti H R and Betke K Schweiz Med W'schr 89 1079 1959
19 Pribilla W Proc. IXth Congress Europ Soc Haemat. Lisbon Karger Basel p 466 1963
20 Rundles R W and Falls H F Am. J Med Sc. 211 641 1946
21 Shahidi N T., Nathan D G and Diamond L K J Clin Invest. 43 510 1964
22 Sjolin S., Wallenius G and Wranne L Acta Haemat 32 239 1964
23 Snijderman E Carreter R and Holt I M Fed Proc 9 371 1950
24 Waldenstrom J Acta Med Scand Suppl 90 380 1937
25 Waldenstrom, J and Kjellberg S R Acta Radiologica 20 618 1939
26 Weatherall, D J The thalassaemia syndromes Blackwell, Oxford 1965
27 Verloop M C., Bierenga M and Diesevaad Njoo A Acta Haemat 27 129 1962
28 Verloop M C Punt K Helleman P W and Bos C C In press
29 Woodrow J C., Wable R L and Martindale J H Med J I 36 1964

Table V Comparison of some data on the patients listed in Tables I to IV

	Presence of "Ringed Siderobl" Bone marrow	Increase of Hb A_2	Increase of Hb F	Presence of Hb H	Erythrocytes with B.C.B[1] inclusion bodies
1 Thalassaemia minor (β thalassaemia)	no	yes	often	no	no
2 α Thalassaemia (Hb H disease)	no	no	no	yes	yes
3 Hereditary sidero achrestic anaemia	yes	no	no	no	no
4 "Thalassaemia like syndrome"	no	no	no	no	no

[1] B C B =Brilliant Cresyl Blue

no increase in haemoglobin A_2 or hae moglobin F

This syndrome most closely resem bles heterozygous thalassaemia It is re-markable, however, that none of the patients examined (Table IV) showed increased haemoglobin A_2 or haemo globin F values Three siblings with the same form of anaemia too, presented no clue indicating α or β thalassaemia

A number of authors (1, 2 6 10) have reported on patients with hypo chromic anaemia and a normal serum iron concentration, in whom neither haemoglobin A_2 nor haemoglobin F was increased But these patients be longed to families with β thalassaemia in which several other members did have an increase of haemoglobin A_2 or haemoglobin F

For the time being, we refrain from classifying the family we described preferring the designation thalassa-emia like syndrome"

Table V presents data on the differ ences between the four disease pictures discussed

Other non-sideropenic hypochromic anaemias

In exceptional cases, hypochromic anaemia not caused by iron deficiency can have yet other causes, some of which we intend to mention without discussing them in detail

Pribilla (19) described a hereditary hypochromic anaemia in which a pa thological haemoglobin fraction was found now known as haemoglobin Koln

There are a few reports on hypo chromic anaemia ascribed to absolute pyridoxine deficiency (8 23)

Shahidi and *Diamond* (21) describ ed a hypochromic anaemia with an increased serum iron concentration with haemosiderosis in the liver and with no stainable iron in the bone marrow

In the case of congenital atransfer rinaemia as described by *Heilmeyer* et al (14), hypochromic anaemia also occurs

Finally chronic infections can in the long run lead to hypochromic anaemia

aplastic anemia group had decreased circulating reticulocytes and a marked reduction of red cell precursors in aspirated marrow

Internal distribution of iron was studied after the intravenous administration of transferrin bound iron (series I) and after the oral ingestion of radioiron (series II) In the intravenous studies 5 to 40 μc Fe^{59} in the form of iron citrate was incubated with normal plasma which had a binding capacity of more than twice the content of iron added When iron was given by mouth 20 to 100 μc were employed depending on the amount of absorption anticipated The form and amount of iron fed is indicated in the individual experiments (Table III) In some individuals both oral and parenteral studies were carried out in order to compare the distribution from the two routes with sufficient time between the two studies to prevent counting interference Ten to 14 days after the administration of radioiron the amount incorporated into the circulating red cell mass was determined from the formula

$$\frac{\text{counts/ml blood} \times \text{blood volume}}{\text{counts injected}}$$

The blood volume was assumed to be 60 ml kg of body weight Plasma iron turnover was calculated according to the formula of Huff *et al* (5) but modified so as to express turnover per 100 ml whole blood (6) according to the simplified formula

In vivo counts were recorded over the sternum at the level of the fourth rib and over the center of liver dullness as determined by the percussion in the right anterior axillary line These counts were carried out at intervals over a period of 14 days after injection Results were expressed as a simple ratio of counts over the liver divided by counts over the sternum It was found that at least 6 days were required in the normal subject for stabilization of the liver sternal ratio In subjects with direct parenchymal uptake of radioiron from the plasma an early equilibrium was obtained but in patients with ineffective erythropoiesis it was found advisable to wait at least 10 days Data illustrative of this are shown in Table I

In vitro counting of blood samples was carried out in a shielded sodium iodide crystal vial counter with a background of approximately 100 c/min *In vivo* counting was carried out with a 2 inch sodium iodide crystal shielded with 2 inches of lead and columnated 2 inches with a 1 inch aperture Plasma iron determinations were made by the method of Bothwell and Mollin (7) and iron binding capacity by the method of Zak (8)

Results

The ferrokinetic measurements on six normal subjects given plasma bound radioiron intravenously are shown in Table II Liver sternum ratios averag-

$$\text{Plasma iron turnover (mg/day/100 ml whole blood)} = \frac{\text{Plasma Iron } (\mu g/100 \text{ ml})}{t\ 1/2 \text{ (minutes)}} \times (100\ \text{hematocrit})$$

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A Study of Internal Distribution of Iron in Man

By Fazle Hosain and Clement A Finch

The movement of radioiron within the body of man may be followed by *in vivo* counting (1, 2 3) Such observations provide a means of detecting abnormalities in red cell kinetics and in iron metabolism Three major pathways of tissue distribution of transferrin bound iron may be illustrated by surface counting (Fig 1) The first and usually the predominant tissue receptor is the erythroid marrow which incorporates radioiron into new erythrocytes that shortly are released to the blood The second also involves the primary uptake of radioiron by the marrow, but with subsequent phagocytosis of the newly formed red cells in various parts of the reticuloendothelial system (ineffective erythropoiesis and hemolytic anemias) The third pattern consists of direct uptake of transferrin bound radioiron by parenchymal cells of the liver It is the concern of this report to establish standards for the practical evaluation of these pathways in man following the intravenous or oral administration of radioiron

Materials and Methods

The individuals studied were divided into the following categories 1) normal, 2) iron deficiency, 3) idiopathic hemochromatosis, 4) ineffective erythropoiesis, and 5) aplastic anemia Normal subjects had hematocrit values of between 40 and 50 per cent and plasma iron levels of 70 to 130 μg/100 ml plasma The diagnosis among subjects in the iron deficiency group was established by the presence of a hypochromic microcytic anemia and a transferrin saturation below 16 per cent (4) The criteria in the idiopathic hemochromatosis group included a near saturation of transferrin the presence of excessive iron deposits in the parenchymal cells of the liver as determined by biopsy, and no specific cause of iron overload Patients with ineffective erythropoiesis showed increase in red cell precursors in the aspirated marrow but normal or depressed circulating reticulocytes Their plasma iron turnover was increased but red cell utilization of radioiron was depressed All patients in the

Table III *Distribution of Radioiron After Oral Injection*

Name	Condition	HCV	SeFe	% Sat	Nature of Dose	Absorption	L/S
Bo	Normal	41	100	32	Trans Fe c̄ Meal	11.0	1.13
Bo		43	130	39	"	6.1	1.22
Ro		49	106	38	1 mg Fe ascorbate	4.5	1.28
Ha		44	108	33	"	12.3	1.35
Be		45	71	28	20 mg Fe ascorbate	2.9	1.33
Gi		46	74	27	100 mg Fe ascorbate	0.5	1.18
Ni		47	81	24		9.1	1.23
Mc	Normal	43	97	27	100 mg Fe citrate	1.7	1.24
Ta		40	97	29		2.1	1.06
An	Iron Deficiency	23	33	8	5 mg Fe ascorbate	28.9	0.63
Ca		27	18	4	"	59.7	0.73
Ar	Idiopathic Hemochromatosis	45	137	47	3 mg c̄ Std Meal	74.0	0.65
We		41	237	97	"	4.3	6.48
Tr	"	44	202	98	"	30.6	2.74
Lo		41	241	100	"	30.5	2.00
Fo		43	246	95	5 mg Fe ascorbate	19.4	1.61
Wa		41	288	97		25.8	1.44
Ar	"	41	298	94	20 mg Fe ascorbate	38.0	1.13
Bo	Idiopathic Hemochromatosis	38	328	83	20 mg Fe ascorbate	58.1	0.73
Ch	"	42	242	95	"	23.2	2.40
Ar	"	45	173	64	100 mg Fe ascorbate	?	1.52
Bo	"	43	257	82	"	?	3.53
Ro	Thalassemia	22.5	153	76	1 mg Fe c̄ Meal	4.8	1.30
Ha	Refractory Anemia	37	200	92	20 mg ascorbate	0.7	6.79

1 to 10 mg iron orally, absorption as judged by the amount of radioactivity appearing in the red cells at two weeks was between 0.5 and 12.5 per cent. Liver sternal ratios varied from 1.13 to 1.33. In two iron deficient subjects there was a 30 per cent and 60 per cent incorporation of the administered radioiron into red cells, and the liver sternal ratios were 0.64 and 0.73. In four patients with idiopathic hemochromatosis given 3 to 5 mg iron orally, liver sternal ratios varied from 0.63 to 6.43. The subject with the low ratio had been depleted of iron by phlebotomy and had a normal serum iron. The highest value clearly fell outside of the ranges obtained after intravenous injection of transferrin bound iron in patients with idiopathic hemochromatosis. One of these patients (Bo) had symptoms of weakness, nausea, and sweating following 100 mg iron as ferrous sulfate and the liver sternal ratio was 3.53 at two weeks. Similar symptoms were seen in a normal subject who had been given 100 mg iron as ferrous ascorbate and was placed in a prone position on his left side for two hours. His plasma iron

Table I *Liver/Sternal Ratios During Two Weeks*

Normal subjects with intravenous Fe^{59}		Thalassemia case with intravenous Fe^{59}		Hemochromatosis patient with oral Fe^{59}	
Days	Ratio	Days	Ratio	Days	Ratio
6	1 01	6	1 35	7	6 42
8	1 14	8	1 47	10	7 90
10	0 96	10	1 70	12	6 51
13	0 93	13	1 73	14	5 08
				17	6 95

ed 1 0, and ranged from 0 85 to 1 13 Between 80 and 85 per cent of the injected radioiron was found in the circulating red cells at two weeks Four patients with iron deficiency anemia were studied Ninety eight per cent of the injected activity localized *in the* circulating red cells, and the liver sternal ratios averaged 0 78 with a range from 0 74 to 0 85 Virtually all injected radioiron was localized in the circulating erythrocyte mass Six patients with idiopathic hemochromatosis in various phases of phlebotomy therapy were studied One of these patients (Ar) had been iron depleted during the preceding year through phlebotomies and despite his high serum iron, had very little excess iron in tissues His per cent utilization was 78, and his ratio was 1 02 The other patients all had some degree of iron overload when studied and their liver sternal ratios ranged from 1 34 to 2 62 Their red cell utilization was 49 to 76 per cent In three patients with ineffective erythropoiesis whose red cell utilization was only 20 to 31 per cent ratios of 1 18, 2 06 and 2 68 were obtained while in three patients with aplastic anemia with over 90 per cent of iron in the tissues ratios of 2 9, 4 2 and 11 0 were observed

Table II *Iron Turnover and Distribution After Intravenous Injection of Plasma-Bound Radioiron*

	Hct	Se Fe	% Sat	% Util	I I I	L/S ratio
Group I — Normal Controls						
Es	49	99	34	85	0 63	0 98
Ha	45	—	40	80	0 79	0 97
Gr	47	93	33	83	0 65	1 13
At	49	125	41	84	0 67	1 03
Pe	46	130	52	81	0 67	0 85
He	44	112	48	81	0 73	1 00
Group II — Iron Deficiency						
Dr	50	27	7	100	0 68	0 85
Mi	69	49	13	100	0 84	0 74
Jo	26	45	11	94	1 59	0 76
Fr	26	29	8	100	1 43	0 81
Group III — Idiopathic Hemochromatosis						
Ch	42	162	81	67	0 94	1 90
Wa	37	258	96	75	1 25	1 61
We	38	225	100	76	1 27	1 34
Ar	45	257	83	78	1 02	1 02
Tr	44	192	85	76	0 99	1 78
Po	48	202	79	49	0 81	2 62
Group IV — Ineffective Erythropoiesis						
Wi	30	180	50	20	4 67	2 68
Wo	25	173	92	22	6 87	1 18
Ba	32	159	87	31	1 97	2 06
Group V — Aplastic Anemia						
Lu	32	158	57	8	0 54	4 23
Si	19	207	95	0	0 40	11 01
Di	22	119	100	<1	0 28	2 90

In the second series of studies, radioiron was administered by mouth and results of this are shown in Table III In normal individuals given from

Table III *Distribution of Radioiron After Oral Injection*

Name	Condition	I CV	SeFe	% Sat	Nature of Dose	Absorption	L/S
Bo	Normal	41	100	32	trans Fe c̄ Meal	11.0	1.13
Bo		43	130	39	"	6.1	1.22
Ro		40	106	38	1 mg Fe ascorbate	4.5	1.28
Ha		44	108	33		12.3	1.35
Be		45	71	28	20 mg Fe ascorbate	2.9	1.33
Ci		46	74	27	100 mg Fe ascorbate	0.5	1.18
Ni		47	81	24		9.1	1.23
Me	Normal	43	97	27	100 mg Fe citrate	1.7	1.24
Ta		40	97	29		2.1	1.06
An	Iron Deficiency	23	33	8	5 mg Fe ascorbate	28.9	0.63
Ca		27	18	4		59.7	0.73
Ar	Idiopathic Hemo chromatosis	45	137	47	3 mg c̄ Std Meal	74.0	0.65
We	"	41	237	97		4.3	6.48
Tr		44	202	98		30.6	2.74
Po		41	241	100	"	30.5	2.00
Lo		43	246	95	5 mg Fe ascorbate	19.4	1.61
Wa		41	268	97		25.8	1.44
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rose to 320 µg/100 ml, and transferrin became saturated. His liver sternal ratio at two weeks was 2.10. Another patient (Ha) with a hyperplastic erythroid marrow, ineffective erythropoiesis and a saturated transferrin, had a ratio of 6.8 after radioiron administration.

Discussion

In these studies, the internal distribution of iron has been evaluated by surface counting over sternum and liver. The former reflects activity in blood and to some extent, in bone marrow, the latter principally reflects the activity localized in parenchymal and Kupffer cells of the liver, though some contribution was also made by blood and overlying rib marrow. The ratio is taken as an index of liver localization of radioiron, although the quantitative limitations on this interpretation are obvious. The determination of the amount of activity localized in the circulating red cells is of additional help in interpreting the ratio.

The kinetics of transferrin bound iron have been described in the normal subject (3). It is known that 80 per cent of radioiron disappears from plasma to reappear in the circulating red cell mass. The exact whereabouts of the remaining 20 per cent is not known. Surface counting can do no more than provide information of the relative concentration of iron in one area as compared with another. In the normal subject there is little evidence of organ localization at a period of two weeks after injection of radioiron when the distribution is reasonably stabilized. Activity over sternum and liver are almost identical. However, when comparison with the iron deficient subject who has virtually no deposition of radioiron in tissues is made, it is apparent that there is some increase in the liver as compared with the sternal area. On the other hand, greater accumulations of radioactivity occur in iron overload states and in certain anemia where there is excessive breakdown of red cells, whether it is defined as ineffective erythropoiesis or hemolysis of young circulating cells.

It has been previously pointed out that liver localization mav represent either direct storage of iron from transferrin in the liver parenchyma or the secondary uptake of iron from defective red cells by the reticuloendothelial tissue of the liver (9). Direct parenchymal uptake occurs in iron overload conditions and in aplastic anemia, whereas reticuloendothelial uptake occurs with breakdown of newly formed red cells. The different time relationships of these two pathways have already been illustrated (Fig. 1) and are not a consideration in the studies reported here. It is of interest here to compare the amount of tissue storage as determined by the per cent utilization of radioiron with the liver sternal ratios (Fig. 2). As might be expected with increased storage the ratio increases. However in those conditions (ineffective erythropoiesis and hemolytic anemia) where tissue storage occurs throughout the entire reticuloendothelial

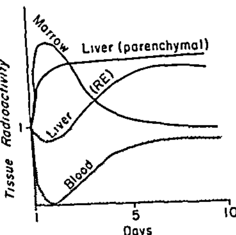


Fig 1 Radioactive iron distribution following intravenous injection of transferrin bound radioiron Activity immediately after injection is taken as 1 Organ counts are determined by in vivo counting The two liver curves indicate uptake directly from transferrin (parenchymal) and from non viable red cells released from the marrow (RE)

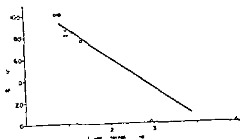


Fig 2 Relationship between body iron distributions (liver/sternal ratio) and amount of iron found in circulating red cells at 2 weeks after intravenous injection of transferrin bound Fe^{59} The open circles are patients with iron deficiency closed circles represent normal subjects squares are idiopathic hemochromatosis triangles are aplastic anemia and crosses are ineffective erythropoiesis

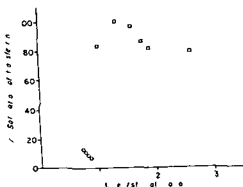


Fig 3 Relationship between body iron distribution and % saturation of transferrin (plasma iron divided by total iron binding capacity) Symbols are the same as in Fig 2

system is compared to parenchymal uptake alone there is proportionately less increase in the liver activity Another relationship examined was that between the serum iron level or per cent saturation of transferrin and the amount of liver iron deposition (Fig 3) It has been postulated (9) that uptake by liver parenchyma might be dependent on a relation between the serum iron level and the transport protein With low plasma iron and low per cent saturation as in iron deficiency or infection nearly all radioiron is localized in the red cell mass When liver uptake is increased a high plasma iron and high transferrin saturation is present However as illustrated by patient Ar who had a normal liver spleen ratio despite a plasma iron of 257 μg/100 ml and a saturation of 83 per cent it appears to be a permissive rather than an obligatory requirement

With these considerations concerning internal iron distribution in mind the distribution of iron administered by mouth may be examined In nor

mal subjects, in patients with iron deficiency anemia and in most patients with idiopathic hemochromatosis, there was no great difference in distribution between oral and intravenous administration This confirms the validity of the double isotope method of measuring iron absorption, where a second isotope of iron was used to correct for the distribution of transferrin bound iron to other than the circulating red cells (10) However, in those instances where a high degree of saturation of transferrin was found, there was sometimes a far greater liver accumulation than would have been anticipated from the intravenous studies Its occurrence in one patient with hemochromatosis, given only 3 mg iron salt along with a test meal suggested that it may occur with normal iron intake These studies are confirmatory of the work of Wheby *et al* who demonstrated in animals (11) and later in man (12) that if iron is infused to the point of saturation of transferrin iron absorbed from the gastrointestinal tract will be deposited directly in the liver rather than being distributed by the transferrin system Iron so absorbed will not be measured by blood sampling techniques and could account for some of the discrepancies in the literature of absorptive measurement between stool and red cell methods It also may explain the lower *normal* values reported with blood sampling techniques in idiopathic hemochromatosis in its advanced stages Such abnormality in distributions may be detected either by monitoring over the liver and sternum as reported here or by total body counting (13)

Summary

Surface monitoring of the liver and splenic areas has been employed as a means of detecting the degree of hepatic storage of radioiron In the present study, data were obtained on subjects with varying disorders affecting tissue distribution of radioiron after intravenous and oral administration In patients with increased parenchymal deposition the liver sternal ratio was increased in general proportion to the amount of tissue deposition A high per cent saturation of transferrin or high serum iron was usually but not invariably associated with an increased deposition of iron in the liver parenchyma Less pronounced liver iron deposition occurred as a part of red cell breakdown within the entire reticuloendothelial system In most subjects distribution after oral iron administration was similar to that after intravenous iron However in some patients with iron overload and a saturated blood transferrin there was evidence of direct hepatic uptake of iron It is concluded that surface monitoring may be employed to determine the general distribution of absorbed iron and whether or not this iron has been distributed by the normal plasma transport mechanism

References

1 Huff R L Elmlinger P J Garcia J F Oda J M Cockrell M D and Lawrence J H Ferrokinetics in normal per

sons and in patients having various erythropoietic disorders J Clin Invest. *50* 1512—1526 1951

2 Pollycove M and Mortimer R. The quantitative determination of iron kinetics and hemoglobin synthesis in human subjects J Clin Invest *40* 753—782 1961

3 Finch C. A Hosain F Morgan E. H., Marsaglia G Giblett E and Hillman R S The ferrokinetic approach to anemia In *Iron Metabolism* Series Haematologica *Vol 6 Munksgaard Copenhagen* 1955 30—49

4 Bainton D F and Finch C A The diagnosis of iron deficiency anemia Amer J Med 37 62—70 1964

5 Huff R L. Hennessy T G Austin R E Garcia J F Roberts B M and Lawrence J H *Plasma and red cell iron* turnover in normal subjects and in patients having various hematopoietic dis*orders*

6 Bothwell T H Hurtado A V Donohue D M and Finch C A Erythrokinetics IV The plasma iron turnover as a measure of erythropoiesis Blood *12* 409—427 1957

7 Bothwell, T H and Mallett, B The determination of iron in plasma or serum Biochem. J 59 599—602 1955

8 Ressler N and Zak B Serum unsaturated iron binding capacity Amer J Clin Path *30 87*—90 1919

9 Hosain Fazle Marsaglia George Noyes Ward and Finch C A The nature of internal iron exchange in man Trans of *the Assn. of Amer Phys 125* (LXXV) 59—63 1962

10 Saylor L and Finch C. A. Determination of iron absorption using two isotopes of *iron* Amer J Physiol *172* 372—376 1953

11 Wheby Munsey S and Jones L G The role of transferrin in iron absorption J Clin Invest 42 1007—1016 1963

12 Wheby Munsey S Effect of transferrin saturation on iron absorption in man New *Eng* J Med *271* 1391—1397 1964

13 Saito Hiroshi Sargent Thornton III Parker Harvard G and Lawrence John H Whole body iron loss in normal man *measured with a gamma spectrometer* J Nuclear Med 5 571—580 1964

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Iron nutrition and iron deficiency

By Lars Garby

The relation between iron nutrition and iron deficiency has long been a matter of considerable discussion The pioneering study of Waldenstrom (1946) is no doubt one of the most important precipitating event in this debate Waldenstrom presented evidence, based on clinical and hematological data that no less than 25 % of women in several communities in the County of Uppland in Sweden were iron deficient If iron deficiency is defined on the basis of hemoglobin values less than 11 to 12 g/100 ml, large survey studies (French 1955, Fry 1961 Kilpatrick 1961, Antila 1962) indicate that iron deficiency is still quite common in many parts of the Western world Most investigators seem to believe that the condition so defined can, in the great majority of instances, be prevented by a fairly moderate increase in the food iron intake

Reluctance in accepting definitions of iron deficiency on the basis of hemoglobin values alone have prompted considerations of data concerning the iron balance, i e intake retention absorption and excretion These data are usually interpreted to show that the iron balance is indeed critical (see for instance Moore 1964 and Bothwell and Finch 1962) Hallberg (1964) added new information on the loss of iron in menstrual blood and by using conventional estimates of iron balance data arrived at the conclusion that more than 25 % of menstruating women in Gothenburg, Sweden are in a status of negative iron balance on an intake of 15 mg of iron per day Since there is good evidence (Soderberg 1958 and 1959 Sterky 1962, see also Blix 1965) that a considerable proportion of women in Sweden have a lower daily intake, these figures predict a very high incidence of iron deficiency

In the present paper some of the basic data of iron balance are critically discussed Reasons are given for the necessity of reevaluating some of these data The results appear to indicate that there is little evidence to suggest that more than a very small proportion of individuals are in a status of negative iron balance when

they are on a daily intake of 10—13 mg of iron

Definition of terms

A scrutiny of the literature reveals the fact that terms like absorption, excretion and retention have been used in a loose way. It will be shown below that a rigorous definition of terms is not only of academic interest. The terms used in the following are defined below.

Retention R = *Intake* I)—*output* (O. This is the equation of material balance resulting from two rate processes I and O of which the I must be assumed to induce perturbation in the system. Experimentally, therefore, only average values over specified time periods at constant intake are meaningful.

Output O is the sum of *fecal output* (O_f), *urinary output* O_u and *menstrual blood flow output* O_m. Other sources of output may be g l ca l l spec al c rcumstances i e sweat d e q d rn al cells

Fecal output O_f = output of unabsorbed iron or iron der d directly from dietary sources O_{fd} plus *iron of body origin* (O_{fb}). The exp r n ntal counterpart of the latter quantity is the output of iron in the faeces at zero intake with the assumption that O_{fb} is independent of I. This assumption is probably true if most of the flow O_{fb} consists of l e ron an l ervl r stes l ut t r ay be false if most of the flow is made up of desquamat l test al ep the al c lls l olved n iron absorpt n

Net transfer A = I − O_{fd}. The experimental counterpart of the al orpt on so defined is I − O with the assumption concerning a n t nsd abs e

T abs pt on is def ned experi ta a the difference between the a u t of rad a ti ty administered orally a l tal rec er d n the faeces during a specified period f oll c on l s relat on to l e al rpt l a sol ut ous The al orp t s let ed l y exper e ts performed a ear l ady state and their interpretation is dependent of the details of the mechanisms by which iron is absorbed. The tracer absorption At on the other hand is defined by experiments in which the details of the mechanisms are essential for a correct interpretation of At in terms of A. The generally accepted model for iron absorption at the cellular level proposed by Crosby (1963) and supported by experimental data by his group (Wentraub, Conrad and Crosby 1964; Conrad, Wentraub and Crosby 1964 and Wheby, Jones and Crosby 1964) visualizes an iron pool in the absorbing epithelial cells situated between the luminal iron and the body proper (plasma). Furthermore, the theory includes the notion of a movement of these cells along the crypts and a final desquamation of the cells at the end of their life span. Depending on the assumptions made concerning the magnitude of the intracellular *iron pool in comparison with the net transfer* and the physical nature of the flow of iron across the two cellular boundaries different situations arise with respect to the interpretation of the observed quantity At. Clearly A may be smaller, equal to or larger than At. Perhaps the most realistic assumptions are 1) that the iron pool is not negligible with respect to the net transfer, 2) that the iron flows across the boundaries are one way flows and 3) that some iron is still present in the cells when they are desquamated. In this case At will be smaller than A.

The data

Carefully controlled chemical balance studies have been performed by Johnston and coworkers (Johnston, Frenchman and Boroughs 1948; Schlaphoff and Johnston 1949; McMillan and Johnston 1951 and Ingalls and Johnston 1954). In these studies the output of faecal iron was recorded at fixed iron intake levels during time periods usually four weeks that ensure a reasonable steady state. In terms of the definitions given above

the relation between the observed quantities O_f and I is

$$O_f = I - A + O_{fb}$$

so that only the difference $A - O_{fb}$ can be evaluated. In all but two of 39 recorded periods of measurements of four weeks in 17 women, this difference was found to be positive at intake levels between 6 and 13 mg per day. At an intake level between 10 and 13 mg per day the mean value from 21 periods in 12 women was 1.6 mg per day (range 0.4—2.7 mg per day). The larger part of the variation is undoubtedly due to experimental error.

Although the difference $A - O_{fb}$ is all that is needed to discuss the retention of iron, we shall proceed to analyze its two components A and O_{fb} in order to inquire into the possible connection between them and the data obtained by studies with radioactive iron. The value of O_{fb} at zero intake was estimated by Ingalls and Johnston (1954) by extrapolating the regression of O_f on I to zero intake, a value of 0.2 mg per day was obtained. If it is assumed that O_{fb} is independant of the intake value, the absorption A is then 1.8 mg per day at these intake levels or 14—18 %. As pointed out above however, the assumption of independancy of O_{fb} on I may not be realistic. Therefore, the average absorption value of 1.8 mg is most probably a minimum value: for example if the faecal output of body iron is instead 0.4 mg per day, the average absorption is 2.0 mg or 15—20 %.

Tracer absorption studies have been carried out by several groups of investigators. The results have been summarized by Moore (1964) who quotes an over all average figure of 6.5 % from 133 experiments on normal subjects receiving labelled food containing between 1 and 17 mg of iron. The variation between individuals is quite large in such studies and its source largely unknown. However it appears highly probable that the difference between the chemical balance absorption figure of 14—18 % and the tracer absorption figure of 6.5 % is significant. There are two possible explanations for this rather remarkable difference. Firstly, as was pointed out above, there is no real theoretical basis for assuming that the tracer absorption figure should be equivalent to the absorption figure derived from a balance study. In fact reasonable assumptions concerning the details of the mechanisms of iron absorption make it probable that the tracer absorption figures are underestimations of the true absorption A. Another explanation for the difference may be found in the fact that the figures from the balance data were obtained from young menstruating female individuals whereas most of the tracer studies have been performed on men. This aspect will be discussed below.

The urinary output of iron amounts to 0.1—0.2 mg per day (Johnston and McMillan 1952, Hallberg 1964). The menstrual blood flow output varies considerably between individuals. Hallberg, Hogdahl, Nilsson and Rybo (see Hallberg 1964) have given the

following figures based upon direct measurements in 450 women who were randomly selected in Gothenburg, Sweden: 50 % lost up to 0.6 mg per day, 75 % lost up to 0.9 mg per day and 90 % lost up to 1.4 mg per day. These figures are in good agreement with those given by Frenchman and Johnston (1949) who found 184 cases and 276 menstrual periods in the literature where direct measurements had been carried out.

The sum of the terms O_B, O_u and O_m is thus 0.4 mg per day in the average man and non-menstruating female and 1.0 mg per day in the average menstruating female. These figures are slightly lower but entirely comparable with the figures estimated by Finch (1964) on the basis of measurements of the decrease in erythrocyte specific activity after intravenous injection of radioiron and of certain assumptions concerning the mixing properties of body iron. Similar estimates have been obtained by using whole body counters to measure the decrease in radioactivity. So far, however, the published studies have failed to meet the requirements of elementary tracer theory that such data can be used to estimate the iron output if and only if 1) the isotope is given in the natural form, i.e. as labelled food, 2) the loss of radioiron is followed to completion, i.e. the total integral is evaluated, and 3) the total body iron content is known.

The balance

It follows from the above calculations that the average menstruating woman retains about 0.6 mg of iron per day on a daily intake of 10—13 mg of iron in a well balanced diet. This amounts to some 200 mg per year. Although the variation of body iron content with time is not known, such a figure is almost certainly not representative for women throughout their life and indicates that many of the individuals studied by Johnston and coworkers were in a period of an unusually large positive retention. Six women were still growing. As judged by haemoglobin, red cell count, serum iron and food history none of the individuals studied had any signs of decreased iron stores. Normal menstrual blood loss was demonstrated in twelve of the women.

The average man and non-menstruating female looses about 0.4 mg of iron per day. An average absorption of 4 % on a daily intake of 10 mg would therefore be sufficient to ensure a non-negative iron balance.

Some 10 % of menstruating women loose more than 1.8 mg per day, i.e. more than the average amount absorbed. Thus 10 % of menstruating females could be expected to be in a situation of negative iron balance. However, this estimate ignores the possibility that the absorption and the faecal output of body iron is dependent on the body iron content when this is within "normal" limits. Since such a dependency has been clearly demonstrated with respect to tracer iron absorption of labelled food when the body iron content is abnormally low, it is reasonable to expect that it exists also within the "normal" limits

the relation between the observed quantities O_t and I is

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The balance

It follows from the above calculations that the average menstruating woman retains about 0 6 mg of iron per day on a daily intake of 10—13 mg of iron in a well balanced diet This amounts to some 200 mg per year Although the variation of body iron content with time is not known such a figure is almost certainly not representative for women throughout their life and indicates that many of the individuals studied by Johnston and coworkers were in a period of an unusually large positive retention Six women were still growing As judged by haemoglobin red cell count serum iron and food history none of the individuals studied had any signs of decreased iron stores Normal menstrual blood loss was demonstrated in twelve of the women

The average man and non menstruating female looses about 0 4 mg of iron per day An average absorption of 4 % on a daily intake of 10 mg would therefore be sufficient to ensure a non negative iron balance

Some 10 % of menstruating women loose more than 1 8 mg per day i e more than the average amount absorbed Thus 10 % of menstruating females could be expected to be in a situation of negative iron balance However this estimate ignores the possibility that the absorption and the faecal output of body iron is dependant on the body iron content when this is within "normal" limits Since such a dependancy has been clearly demonstrated with respect to tracer iron absorption of labelled food when the body iron content is abnormally low it is reasonable to expect that it exists also within the "normal" limits

It may therefore be concluded that the available data concerning the iron balance do not support the idea that iron deficiency is likely to develop in menstruating females on a food iron intake of some 10—13 mg per day

Summary

The available data concerning the iron balance, i e absorption, excretion and retention, is critically discussed

Reasons are given for the necessity of re evaluating some of the data

The results appear to indicate that, contrary to currently expressed ideas there is little evidence to suggest that more than a very small proportion of individuals are in a status of negative iron balance on a daily intake of 10—13 mg of iron

References

Antila V Acta Med Scand Suppl 393 *173* 1963

Blix G Acta Soc Med Upsal *70* 117 1965

Bothwell T H and Finch C A "Iron metabolism" *Little Brown and Co* Boston 1962

Conrad M E Weintraub L R & Crosby W H J Clin Invest *43* 963 1964

Crosby, W H Blood *22* 441 1963

Finch C A J Clin Invest *38* 392 1959

French, D G Proc Roy Soc Med *48* 347 1955

Frenchman R & Johnston F A J Am Diet Ass 25 217 1949

Fry J Brit Med J *2* 1732 1961

Hallberg L Naringsforskning *8* 1 1964

Ingalls R L & Johnston F A J Nutr (U S A) *53* 351 1954

Johnston F A, Frenchman R & Boroughs, E D J Nutr (U S A) *35* 453 1948

Johnston F A & McMillan T J J Am Diet Ass *28* 633 1952

Kilpatrick G S Brit Med J *2* 1736 1961

McMillan T J & Johnston F A J Nutr (U S A) *44* 383 1951

Moore C V In F Gross "Iron Metabolism p 241 Springer Berlin 1964

Schlaphoff, D M & Johnston F A J Nutr (U S A) *39* 67 1949

Sterky G Acta Paediat Suppl 135 *51* 185 1962

Soderberg S Soc med Tidskr *35* 3 1958

Söderberg S Soc med Tidskr *36* 143 1959

Waldenström J Acta Med Scand Suppl *170* 252 1946

Weintraub L R Conrad M E & Crosby W H Blood *24* 19 1964

Wheby M S Jones I G & Crosby W H J Clin Invest *43* 1433 1964

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Iron Absorption after Partial Gastrectomy

A comparative study on the absorption from ferrous sulphate and hemoglobin

By Leif Hallberg Lennart Sölvell and Bengt Zederfeldt

Iron deficiency anemia is common after partial gastrectomy — it is more common in women than in men and more common after operations of the Billroth II type than after the Billroth I type (1 5)

The main causes of the postgastrectomy iron deficiency anemia discussed are (1) blood loss (2) reduced intake of food iron and (3) impaired absorption of iron from the food Gastrointestinal occult blood loss is probably not a main cause of the anemia as the incidence of positive benzidine reactions was the same in anemic and non anemic patients after partial gastrectomy as in non gastrectomized peptic ulcer patients (1) Moreover the loss of body radioiron over a period of one year was the same in 6 gastrectomized patients (of whom 3 were anemic) as in normals (10) Both in men and in women a lower intake of iron with food was observed in anemic than in non anemic gastrectomized patients However a low intake of iron cannot be the sole explanation of the anemia as in anemic men the average daily intake of iron was 11 5 mg This amount should be sufficient to balance the small daily losses of iron in men

Because of this the main attention has been given to the ability to absorb iron after partial gastrectomy

The absorption of iron from iron salts seems to be unaffected by gastrectomy (1 9) and it has repeatedly been shown that iron deficiency anemia after partial gastrectomy responds to oral iron therapy (11) A marked reduction of the absorption has been observed in most studies on food iron absorption (2 4 10) However in a recent study on food iron absorption measured by adding an inorganic radioiron tracer to a meal the effect of gastrectomy was very slight (7)

The great variation in the absorption of iron between and within individuals makes it difficult to determine if the absorption is affected by the gastrectomy or not in the single indi

Table I *Absorption of Iron from Ferrous Sulphate and Hemoglobin in Normals, Blood Donors, and Patients with Iron Deficiency Anemia*

	Case	Age	Sex	Hb conc g/100 ml	MCHC (per cent)	Iron Absorption (per cent) HbFe	$FeSO_4$	Absorption ratio FeHb/$FeSO_4$
Normals	N 1	23	M	15.2	32.3	4.9	5.0	0.98
	N 2	25	M	14.5	33.0	4.6	3.4	1.35
	N 3	23	M	13,7	32.6	2.9	3.0	1.0[illegible]
	N 4	24	M	13.1	31.2	4.5	3.3	1.33
								Mean 1.1[illegible]
Blood Donors	BD 1	49	M	15.6	31.8	27.4	60.7	0.45
	BD 2	49	M	15.5	33.0	10.9	35.3	0.31
	BD 3	48	M	14.4	31.3	8.7	48.2	0.18
	BD 4	49	M	13.6	31.6	25.9	62.9	0.41
Iron Def Anemia	A 1	71	F	11.4	30.0	13.5	47.5	0.[illegible]
	A 2	39	F	10.5	30.0	14.6	32.1	0.46
	A 3	54	F	9.3	29.1	10.0	40.0	0.25
								Mean 0.34

vidual. When studying the effect of gastrectomy on the absorption of iron salts and of food iron it is thus important to make comparative absorption studies in the same subject. Such comparisons are greatly facilitated by using a double radioiron technic. In the present study a comparison was made of the absorption of iron from ferrous sulphate and from hemoglobin which were labelled with two different radioiron isotopes and administered on alternate days to the same subject. The study included two groups of patients with Billroth I or Billroth II gastrectomy as these two operations differ significantly in their tendency to develop iron deficiency anemia. A group of non-gastrectomized subjects was also studied in the same way to get a firmer basis for the evaluation of the effect of gastric resection on the absorption of iron.

Methods

A double radioiron technic with the same experimental design as previously described was used (3). Five milligram of iron was given orally every morning for 10 days after an overnight fast. Solutions of ferrous sulphate and solutions of hemoglobin (hemolyzed red cells) labelled with different radioiron isotopes (Fe^{55} and Fe^{59}) were given on alternate days. Each dose (25 ml) also contained 5 mg ascorbic acid and 10 ml 70 per cent black currant syrup. Two weeks after the last oral dose a blood sample was drawn for determination of Fe^{55} and Fe^{59}. The details of the method were the same as previously described.

The ferrous sulphate was labelled with Fe^{55} and the hemoglobin with Fe^{59}. The labelled hemoglobin was prepared by giving 500—1000 µC of $FeCl_3$ (about 15 µC/µg Fe) intravenously to a rabbit. One week later the rabbit was exsanguinated by carotid artery bleeding. The red cells were hemolyzed by freezing and thawing. Each dose of "hemoglobin iron" contained about 4 µC Fe^{59} and was diluted with non-radioactive frozen and thawn rabbit red cells to obtain 5 mg of elemental iron in each dose.

Table II Absorption of Iron from Ferrous Sulphate and Hemoglobin in Patients with Partial Gastrectomy (Billroth I)

Case	Age	Type of ulcer	Hb conc. g/100 ml	MCHC (per cent)	Iron absorption (per cent) HbFe	FeSO4	Absorption ratio (FeHb FeSO4)
1	4o	D	1o3	31 9	3 6	3	0 99
2	oo	D	1o 2	32 3	3 7	2 9	1 2o
3	4)	D	14 7	32 0	6 0	o 7	1 0o
4	43	D	14 1	32 0	2 0	2 1	0 96
5	o7	G	13 6	32 4	6 3	21 0	0 30
6	49	G	13 5	31 0	7 9	17 4	0 4o
	4)	D	1?3	31 1	2 7	?6	1 02

(Gastric D=Duodenal

Table III Absorption of Iron from Ferrous Sulphate and Hemoglobin in Patients with Partial Gastrectomy (Billroth II)

Case	Age	Type of ulcer	Hb conc. g/100 ml	MCHC (per cent)	Iron absorption (per cent) HbFe	FeSO4	Absorption ratio (FeHb FeSO4)
1	46	D	14 1	?9 4	12.o	13 1	0 9o
2	44	D	13.o	31 4	16 6	20.5	0 81
3	47	D+G	13 2	?8 7	15	14 1	1 12
4	40	D	1?9	29 7	.5	3 1	2 43
5	48	D	1?1	28 1	9.3	o 7	1 64
6	oo	G	11 1	27 8	o 9	3 9	1.o4

G=Gastric D=Duodenal

Material

Thirteen male gastrectomized patients were included in the study. In all patients a partial gastrectomy was done about 3 years ago for ... duodenal or gastric ulcer. Seven patients were operated according to Billroth I ... patients according to Billroth II (see Table II and III). None was anemic at the ... of the operation and gastrointestinal bleeding was in no case the indication for the ... in ... After the operation there was no recurrent ulcer nor bleeding and no iron therapy was given. The blood loss at the operation was not quantitated but estimated ... reported to the 1-3 pints of blood ... during the operation.

Two groups of non-gastrectomized subjects were also studied: one group consisted of 4 healthy male medical students (N), the other one of subjects with iron deficiency. The latter group comprised 4 healthy non-anemic male blood donors (BD) who had given 5—68 pints of blood during the last 15 years (see Table I) and who had never had any iron supplementation. The group also included 3 patients with iron deficiency anemia (A).

Results

A Non-gastrectomized subjects

As shown in Table I the absorption of iron from ferrous sulphate and from hemoglobin was about the same in the 4 normal subjects. In the iron deficient

group, there was an increase in the absorption of iron from both compounds but a markedly greater increase in the absorption from ferrous sulphate than from hemoglobin giving an absorption ratio (FeHb/$FeSO_4$) of about 0 3

B *Partially gastrectomized subjects*

1 *Billroth I group* The results from the 7 male patients operated according to Billroth I are given in Table II In the 4 subjects with a hemoglobin concentration above 14 g/100 ml blood the absorption of iron from ferrous sulphate and from hemoglobin was about the same and of the same magnitude as observed in the normal subjects

In subjects 5 and 6, who had a hemoglobin concentration below 14 g/100 ml blood there was an increased absorption of iron from ferrous sulphate and possibly a somewhat higher absorption of hemoglobin iron compared to subjects 1—4 Subject 7 showed a low absorption both from ferrous sulphate and from hemoglobin However this subject differed in one respect from all the others by having evident dumping symptoms each time the sugar containing iron solution were taken

2 *Billroth II group* The results from the 6 male patients operated according to Billroth II are given in Table III Judging from the MCHC values, they were all iron deficient and all but one had hemoglobin values below 14 g/100 ml blood The three subjects (4—6) with the lowest hemoglobin values had a markedly lower absorption from both ferrous sulphate and hemoglobin than subjects 1—3

Discussion

It is difficult to study the effect of gastrectomy on the absorption of iron. The difficulty cannot be overcome by studying the same patient before and after the operation as e g the size of the iron stores on the two occasions is not known In the present study, the effect of gastrectomy on the iron absorption was analyzed not only by comparing the iron absorption from ferrous sulphate and hemoglobin in the same patient but also by comparing the absorption ratio (hemoglobin iron/ferrous iron) with the ratio in non gastrectomized subjects — normals and subjects with various degrees of iron deficiency The interpretation of the results in the gastrectomized patients was greatly facilitated by the following observations (1) in normal subjects, the absorption of hemoglobin iron and ionized iron was low and about the same (2) in non gastrectomized iron deficient subjects, the absorption was increased from both types of iron compounds but significantly more from the ionized iron

In the Billroth II group the markedly increased absorption of hemoglobin iron in subjects 1—3 was probably due to an iron deficiency In the iron deficient non gastrectomized subjects the absorption of iron from ferrous sulphate was about 3 times higher than from hemoglobin However in these Billroth II subjects (1—3) the absorption of iron from ferrous

sulphate was increased only up to the same level as the absorption from hemoglobin. Thus the absorption of iron from ferrous sulphate was more impaired than that from hemoglobin. In subjects 4—6 who had the lowest hemoglobin values of the Billroth II group the absorption from both hemoglobin and ferrous sulphate was evidently reduced compared with subjects 1—3. The reduction was most marked for ferrous sulphate giving an absorption ratio (FeHb/FeSO$_4$) much above 1 against an expected ratio of about 0.3 found in non gastrectomized iron deficient subjects (Table I).

It may thus be concluded that after the Billroth II gastric resection the absorption from an iron salt was reduced in all subjects and more reduced in the subjects with the lowest hemoglobin values. The absorption from ferrous sulphate was more reduced than the absorption of hemoglobin iron, which was quite evident only in the subjects with the lowest hemoglobin values.

Thus there is a fundamental difference between non gastrectomized iron deficient patients who absorb more iron the more severe the iron deficiency and patients with a Billroth II gastric resection who show an inverse relationship between absorption and degree of iron deficiency. The iron deficiency anemia after a Billroth II resection thus reflects the degree of the absorption defect for iron induced by the operation. However additional factors influence the rate of development of an iron deficiency after a gastric resection, e.g. the amount of stored iron before the operation, the amounts of blood lost and transfused at the operation, other iron losses (menstruations etc.) and the intake of iron from diet or medication. All such factors as well as the time interval to the operation have to be considered in comparative studies of the effect of various resection methods on the iron balance. In the selection of the present material attempts were made to consider most of these factors.

The Billroth I gastric resection was followed by a less severe impairment of the iron absorption than the Billroth II resection. In subjects 1—4 with normal hemoglobin values in the Billroth I group the absorption of iron from ferrous sulphate and from hemoglobin was of the same magnitude as in the normal non anemic subjects and the absorption ratio (FeHb/FeSO$_4$) was about 1 as in the normal subjects. In subjects 5 and 6 who had subnormal hemoglobin values the iron absorption was increased but not to the same extent as in the non anemic iron deficient blood donors. This indicated that there was a minor defect of the absorption of iron in the anemic Billroth I patients. However, contrary to the Billroth II material the absorption ratio (FeHb/FeSO$_4$) was about the same in the Billroth I patients with subnormal hemoglobin values as in the non gastrectomized iron deficient patients. The very low absorption in subject 7 was probably related to the mentioned dumping symptoms with rapid gastric emptying and rapid transfer of the intestinal contents.

The present study thus indicates that the Billroth I gastric resection may give a minor quantitative absorption defect affecting the iron absorption from both hemoglobin and iron salts Such a quantitative, but more pronounced defect was observed also after the Billroth II resection This may be explained by for instance a shorter time of digestion in the stomach, a lower production of hydrochlorid acid, and a more rapid transport of the intestinal contents

The observation of a greater impairment of the iron absorption after the Billroth II than after the Billroth I gastric resection is consistent with the clinical observation that there is a higher frequency of iron deficiency anemia after the Billroth II resection The main discrepancy compared to previous iron absorption studies is the reduced absorption from ferrous sulphate observed after the Billroth II resection (2 9) The most probable explanation of this discrepancy is the previously mentioned difficulty to establish that the absorption is changed by gastrectomy in the single subject

The two types of gastric resection affected the iron absorption differently After both types a reduction of the iron absorption from hemoglobin and from ferrous sulphate was noted (a quantitative defect) After the Billroth II resection a qualitative defect was also observed, i e the marked reduction of the absorption from ferrous sulphate The pathophysiological basis of this difference between the Billroth I and Billroth II gastric resection is probably the exclusion of duodenum and a part of the jejunum with the Billroth II resection Such an exclusion may impair the absorption from an iron salt more than of hemoglobin iron as iron salts are absorbed in the upper part of the intestine in ionized form and thus dependent on pH, while hemoglobin iron is absorbed as an iron porphyrin complex which very likely is unaffected by pH (6 8)

Summary

The iron absorption from ferrous sulphate and hemoglobin was compared in the same subject using a double radioiron method The study was made in male subjects with a Billroth I or with a Billroth II gastric resection and in a material of non gastrectomized normal or iron deficient subjects

A quantitative absorption defect involving both hemoglobin iron and ionized iron was observed in patients with both types of gastric resection The defect was most pronounced after the Billroth II resection After this operation there was also a qualitative absorption defect which affected the absorption from iron salts

The absorption defects seemed to be more pronounced the more severe the iron deficiency indicating a causal connection

The observed differences in degree and type of impairment of the iron absorption probably explain the different frequency of iron deficient anemia after the two types of gastric resection

References

1 Baird I M Blackburn E K and Wilson G M The pathogenesis of anaemia after partial gastrectomy I Development of anaemia in relation to time after operation blood loss and diet Quart J Med N S 28 21—34 1959

2 Baird I M, and Wilson G M The pathogenesis of anaemia after partial gastrectomy II Iron absorption after partial gastrectomy Quart J Med N S 28 35—41 1959

3 Brise H and Hallberg L Iron Absorption Studies II Acta med scand suppl 3 6 to vol 171 1962

4 Choudhury M R and Williams J Iron absorption and gastric operations Clin Sci 18 527—532 1959

5 Deller D J and Witts L J Changes in the blood after partial gastrectomy with special reference to vitamin B_{12} I Serum vitamin B_{12}, haemoglobin serum iron and bone marrow Quart J Med 31 71—88 1962

6 Hallberg L and Solvell L Absorption of hemoglobin iron in man Abstract Xth Congress of the International Society of *Haematology* 1964

7 Turnbull A Absorption of iron from food after partial gestrectomy Abstract Xth Congress of the International Society of Haematology 1964

8 Turnball A, Cleton F and Finch C A Iron Absorption IV The absorption of hemoglobin iron J Clin Invest 41 1897—1907 1962

9 Smith M D and Mallet B Iron absorption before and after partial gastrectomy Clin. Sci 16 23—34 1957

10 Stevens A R Pirzio Biroli G Harskins H N Nyhus L M and Finch C A Iron absorption in patients after partial gastrectomy Ann Surg 149 534—538, 1959

11 Witts L J Anemia and the alimentary tract Sydney Watson Smith Lecture 1955 Royal College of Physicians Edinburgh

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The absorption defects seemed to be more pronounced the more severe the iron deficiency, indicating a causal connection.

The observed differences in degree and type of impairment of the iron absorption probably explain the different frequency of iron deficient anemia after the two types of gastric resection.

addition to a number of other factors The enzyme of catalase containing iron in the molecule as prosthetic group is counted among these enzymes

In view of the observation that desferrioxamine B in addition to eliminating superfluous iron from the organism also produces a stimulating effect upon oxidative metabolic processes (12) and in consideration of the role of iron as catalyser in the electron transfer in these processes the effect of desferrioxamine B upon the enzymatic peroxidative activity of catalase in comparison with the level of serum iron was investigated in vivo and in vitro in this study Parallel investigations in the effect of the compound upon the enzymatic oxidative activity of PPD oxidase containing copper in the prosthetic group and ranged with catalase in the group of oxidoreductase were carried out at the same time

Material and Method

Investigations in vivo in the first series of tests were carried out by [illegible] cases of intravenous administration of desferrioxamine B in 9 subjects i.e. 3 with reduced 3 with normal and 3 with increased serum iron values Blood samples were collected before and [illegible] minutes [illegible] 3 [illegible] and 24 hours after the application The values of iron bound to transferrin the unsaturated iron binding capacity (UIBC) and serum copper were determined in these samples as were the plasma catalase and PPD oxidase activities Two patients suffering from haemochromatosis and two healthy adults as controls were subjected to test in an analogous manner Blood samples in the [illegible] series of tests were taken at [illegible] intervals

Desferrioxamine B: Desferal, Ciba AG, Basel, Switzerland

equal to those in the first series in addition to transferrin bound iron, UIBC and serum copper and enzymatic catalase and PPD oxidase activities we also determined the quantity of total serum iron (iron bound to transferrin together with iron bound to desferrioxamine B)

Investigations in vivo were preceded by investigations in vitro performed in such manner that trivalent iron desferrioxamine B compound and ferrioxamine compound were added to native serum Analysis of the enzymatic activity of catalase and PPD oxidase was performed in the native serum as well as in the three groups of loaded sera

Determination of transferrin bound iron was performed according to the method of Heilmeyer and Plotner (5) copper analyses were made with the modification according to Huyer et al (6) the method of Diehl (3) was applied in measuring the enzymatic activity of plasma catalase and the Keler Baťoka (10) modification was employed in determining the oxidative activity of serum PPD oxidase. The determination of total serum iron (transferrin bound iron together with iron bound to desferrioxamine) was carried out according to Kelerle (8) with the modification that the serum in acid medium was placed for the duration of 15 minutes into a waterbath at 100 C. The determination of unsaturated iron binding capacity (UIBC) was performed with modification by Ventura (11)

Experiments and Results

Investigations in vitro

In order to exclude the possibility of the compounds desferrioxamine B and ferrioxamine and trivalent iron affecting the enzymatic activity of catalase and PPD oxidase the experiments in vitro were performed so that the native serum was loaded with each of the compounds in such manner that the final concentration amounted to 500 400 300 200 and 100 mg in 100 ml of

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The Effect of Desferrioxamine B upon the Enzymatic Catalase and PPD-Oxydase Activity in Plasma and Serum

By A. Hahn and Mira Keler-Bacoka

Administration of the compound desferrioxamine B for the scope of specific and selective elimination of iron from oversaturated organs and tissues (1) has lately found wide application in the treatment of various pathologic conditions requiring removal of superfluous iron from the organism. Data have been presented on treatment with the compound administered in cirrhosis of the liver, essential pulmonary siderosis, sideroachrestic anemia, transfusion haemosiderosis and in iron poisoning; papers describing the administration of the compound in the treatment of haemochromatosis (2, 4, 7, 9) are particularly numerous.

In addition to excretion and elimination of large quantities of iron — up to 90 mg in 24 hours — by way of the urine, clinical improvement in some patients was observed to set in much earlier than could be explained in direct relation with the quantity of iron eliminated (12). The very quick depigmentation of the skin was particularly notable: it would take place as early as two to 3 weeks after treatment was started, although the tissues are then still oversatured with iron in haemochromatosis. The examination of excised skin has shown that the treatment reduced the melanin content in the basic layer of the skin. Wohler (12) has tried to interpret the phenomenon as probably being the catalytic effect of desferrioxamine B in the sense of inhibition of adrenaline oxidation through adrenochrome to melanin in presence of trivalent iron which mobilized by means of the compound from the tissue during treatment occurs in increased quantities in the circulating plasma. Wohler also believes that the rapid subjective improvement of the patient's condition in the initial stage of treatment could be related not only to the elimination of the superfluous iron but also to the acceleration of metabolic oxidative processes. Iron performs a catalytic function in a function in a series of reactions of redox type in the sense of electronic transfer: enzymes which catalyse redox processes are known to play an essential part in these processes in

addition to a number of other factors. The enzyme of catalase containing iron in the molecule as prosthetic group is counted among these enzymes.

In view of the observation that desferrioxamine B, in addition to eliminating superfluous iron from the organism, also produces a stimulating effect upon oxidative metabolic processes (12) and in consideration of the role of iron as catalyser in the electron transfer in these processes, the effect of desferrioxamine B upon the enzymatic peroxidative activity of catalase in comparison with the level of serum iron was investigated in vivo and in vitro in this study. Parallel investigations in the effect of the compound upon the enzymatic oxidative activity of PPD oxidase containing copper in the prosthetic group and ranged with catalase in the group of oxidoreductase were carried out at the same time.

Material and Method

Investigations in vivo in the first series of tests were carried out by means of intravenous administration of desferrioxamine B[1] in 9 subjects, i.e. 3 with reduced, 3 with normal and 3 with increased serum iron values. Blood samples were collected before and 5 minutes, 1, 3, 5 and 24 hours after the application. The values of iron bound to transferrin, the unsaturated iron binding capacity (UIBC) and serum copper were determined in these samples as were the plasma catalase and serum PPD oxidase activities. Two patients suffering from haemochromatosis and two healthy adults as controls were subjected to tests in an analogous manner. Blood samples in this series of tests were taken at intervals equal to those in the first series. In addition to transferrin bound iron, *UIBC and serum* copper and enzymatic catalase and PPD oxidase activities we also determined the quantity of total serum iron (iron bound to *transferrin together with iron bound to des*ferrioxamine B).

[1] Desferrioxamine B (Desferal) Ciba AG Basel Switzerland

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In view of the observation that desferrioxamine B in addition to eliminating superfluous iron from the organism also produces a stimulating effect upon oxidative metabolic processes (12) and in consideration of the role of iron as catalyser in the electron transfer in these processes the effect of desferrioxamine B upon the enzymatic peroxidative activity of catalase in comparison with the level of serum iron was investigated in vivo and in vitro in this study Parallel investigations in the effect of the compound upon the enzymatic oxidative activity of PPD oxidase containing copper in the prosthetic group and ranged with catalase in the group of oxidoreductase were carried out at the same time

Material and Metho l

I est gat ons in o n tle f rst ser es f tests were carr e l out l y n ea s f i tra e o s adn n trat on of desferr oxa ne B n 9 subjects e 3 w l re luce l 3 w tl or al and 3 w tl crease l seru i ron alues Bloo l san ples were collecte l l ef re an l o n nutes 1 3 o an l 24 l ours after tle appl cat n Tl e values of iron l ound to tra s f rr tl e u aturated ro l n l n capa ty UIBC and seru c pper were let r l tl e e san ples as were tl e plas a catalase and serum PPD ox lase act les Two pat e ts suffer ng from l aem l ron atos s an l two l eal l y adults as co trols were sul jecte l to te ts l a s n ialoguous i an er Bloo l sa ples in tl s er s f te ts were tak n at inter als equal to tl ose in tl e f rst ser es n ad l tion to transferrin l ound iron UIBC an l serum copper an l enzymat c catalase an l PPD ox dase activit es we also determ ned tl e quant ty of total s rum iro (ron l ound to transferr n togetl er w th iron l ound to les ferrioxamine B)

In vest gat ons vivo were prece led l y invest gations in vitro performed in such n anner tl at tr alent iron desferr oxam ne B con p un l and ferr oxam ne compound were added to native seru Analys s of tl e enzy i at c activ ty of catalase an l PPD ox lase as perf rn ed n tl e nat ve s rum as well as n tl e tl ree grou ps of loaded sera

Determ nat on of transferrin l ou l ron was performed accord ng to tl e metl od of He l n eyer an l I lotner (a) copper analyses were made w tl tl e mod f cat on accord ng to Hoyer et al 6) tl e metl od of D lle (3) was appl e l m asur ng tl e enzymat c activity of plasn a catalase an l tl e Keler Bač ka (10) mod f cat on was employed in determ ning tl e ox dative act ity of serum PPD oxidase Tl e determ nat on of total seru n iron (transferrin l o nd ir n togetl er w th iron boun l to des f rr oxan ne was carr ed out accord ng to Keberle 8 w th the r od fication tl at tl e serum i ac l n e l um was place l for tl e lurat on of 15 m nutes nto a waterl ath at 100 C Tl e leterm nat o of unsaturate l iron l nd ng capac ty UIBC was performe l w th n od f cat on l y Ventura (11

I xperimen ts an l Results

Investigations in vitro

In order to exclude the possibility of the compounds desferrioxamine B and ferrioxamine and trivalent iron affecting the enzymatic activity of catalase and PPD oxidase the experiments in vitro were performed so that the native serum was loaded with each of the compounds in such manner that the final concentration amounted to 500 400 300 00 and 100 mg in 100 ml of

Desferr oxa e B Desferal C ba AG Ba l Sw tzerland

Department of Medicine University Hospital Zagreb
Head Prof A Hahn M D

The Effect of Desferrioxamine B upon the Enzymatic Catalase and PPD-Oxydase Activity in Plasma and Serum

By A Hahn and Mira Keler Bacoka

Administration of the compound desferrioxamine B for the scope of specific and selective elimination of iron from oversatured organs and tissues (1) has *lately found* wide application in the treatment of various pathologic conditions requiring removal of superfluous iron from the organism Data have *been* presented on treatment with the compound administered in cirrhosis of the liver, essential pulmonary siderosis, sideroachrestic anemia transfusion haemosiderosis and in iron poisoning, papers describing the administration of the compound in the treatment of haemochromatosis (2, 4 7, 9) are particularly numerous

In addition to excretion and elimination of large quantities of iron — up to 90 mg in 24 hours — by way of the urine, clinical improvement in some patients was observed to set in much earlier than could be explained in direct relation with the quantity of iron eliminated (12) The very quick depigmentation of the skin was particularly notable it would take place as early as two to 3 weeks after treatment was started, although the tissues are then still oversatured with iron in haemochromatosis The examination of excised skin has shown that the treatment reduced the melanin content in the basic layer of the skin Wöhler (12) has tried to interpret the phenomenon as probably being the catalytic effect of desferrioxamine B in the sense of inhibition of adrenaline oxidation through adrenochrome to melanin in presence of trivalent iron which, mobilized by means of the compound from the tissue during treatment occurs in increased quantities in the circulating plasma Wohler also believes that the rapid subjective improvement of the patient s condition in the initial stage of treatment could be related not only to the elimination of the superfluous iron but also to the acceleration of metabolic oxidative processes Iron performs a catalytic function in a function in a series of reactions of redox type in the sense of electronic transfer enzymes which catalyse redox processes are known to play an essential part in these processes, in

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Material and Method

Investigations in vivo in the first series of tests were carried out by means of intravenous administration of desferrioxamine B[1] in 9 subjects i e 3 with reduced 3 with normal and 3 with increased serum iron values Blood samples were collected before and 5 minutes 1 3 5 and 24 hours after the application The values of iron bound to transferrin the unsaturated iron binding capacity (UIBC) and serum copper were determined in these samples as were the plasma catalase and serum PPD oxidase activities Two patients suffering from haemochromatosis and two healthy adults as controls were subjected to tests in an analogous manner Blood samples in this series of tests were taken at intervals equal to those in the first series In addition to transferrin bound iron UIBC and serum copper and enzymatic catalase and PPD oxidase activities we also determined the quantity of total serum iron (iron bound to transferrin together with iron bound to desferrioxamine B)

Investigations in vivo were preceded by investigations in vitro performed in such manner that trivalent iron desferrioxamine B compound and ferrioxamine compound were added to native serum Analysis of the enzymatic activity of catalase and PPD oxidase was performed in the native serum as well as in the three groups of loaded sera

Determination of transferrin bound iron was performed according to the method of Heilmeyer and Plotner (5) copper analyses were made with the modification according to Hoyer et al. (6) the method of Dille (3) was applied in measuring the enzymatic activity of plasma catalase and the Keler Bačoka (10) modification was employed in determining the oxidative activity of serum PPD oxidase The determination of total serum iron (transferrin bound iron together with iron bound to desferrioxamine) was carried out according to Keberle (8) with the modification that the serum in acid medium was placed for the duration of 15 minutes into a waterbath at 100 C The determination of unsaturated iron binding capacity (UIBC) was performed with modification by Ventura (11)

Experiments and Results

Investigations in vitro

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[1] Desferrioxamine B (Desferal) Ciba AG Basel Switzerland

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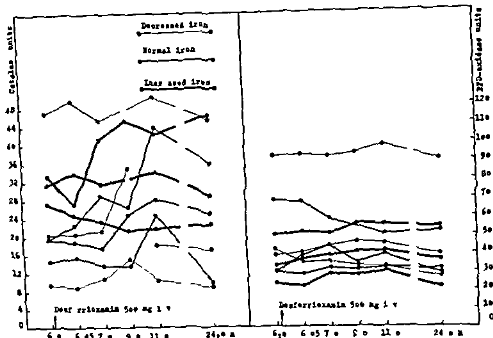


Table 2 Values of enzymatic activity of catalase and PPD oxidase in plasma and serum prior to and after i v application of Desferrioxamine B in subjects with different initial serum iron values

The results of the determination of PPD oxidase activity — as seen in table 2 — showed no essential change after application

Two patients with haemochromatosis (table 4) and two normal subjects as controls (table 3) were treated in analoguous manner in the second series of experiments Determination of total serum iron i e transferrin bound iron together with iron bound to desferrioxamine B was additionally performed in this series of experiments The results of the control tests (table 3) and in part also the results obtained in two patients with haemochromatosis (table 4) show analogy with the results of the first series of experiments i e a marked reduction of transferrin bound iron 5 minutes after application and practically unchanged values of serum copper and PPD oxidase after application The values of total iron after administration of desferrioxamine B underwent no essential change in the control tests but showed marked increase in the patients with haemochromatosis A notable increase in catalase activity after the application regardless of the concentration of total iron and of transferrin bound iron was registered in both the patients and the controls Equal to the first series of tests the control tests in the second series showed an obvious rise in UIBC values immediately on the ad

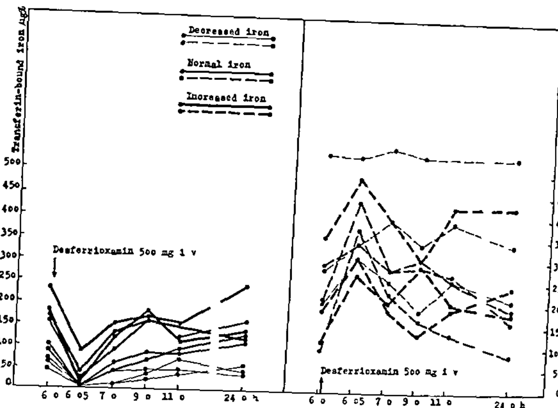


Table 1 Values of transferrin bound iron and unsaturated iron binding capacity (UIBC) in the serum prior to and after i v application of the compound Desferrioxamine B 500 mg in subjects with different initial values of serum iron

serum Examination of the enzymatic activities of catalase and PPD oxidase showed no essential difference between the native serum and the loaded one

Investigations in vivo

The results of the first series of experiments, presented in table 1, showed that a marked reduction of transferrin bound iron had taken place in all examined subjects 5 minutes after application of the compound Furthermore in the subjects with reduced initial serum iron values the serum iron became completely bound to desferrioxamine B so that the determinations in these subjects showed nil values 5 minutes after administration Simultaneously there was an obvious increase in all examined subjects of the unsaturated iron binding capacity (UIBC) reflecting the condition of serum transferrin free from iron The level of serum copper underwent no essential change in the course of the experiment

The values of enzymatic catalase activity presented in table 2 indicate that enzymatic activity increased in 6 of the investigated subjects 1 3 or 5 hours after the application regardless of the serum iron level but these values returned to the range of initial results after 24 hours In three instances catalase activity remained practically unchanged throughout the experiment

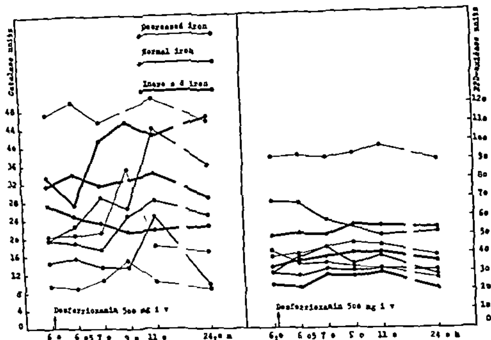


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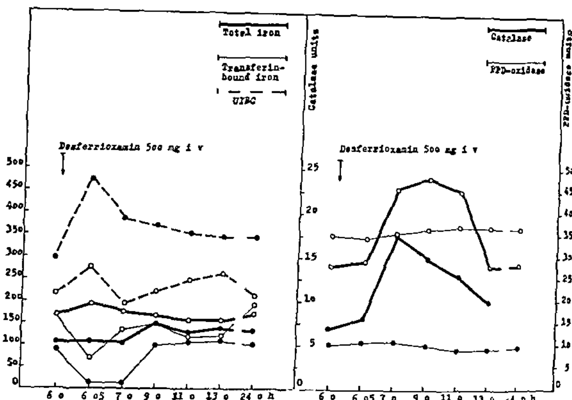


Table 3 Values of total serum iron transferrin bound iron and UIBC and enzymatic activity of catalase and PPD oxidase prior to and after i v application of Desferrioxamine B 500 mg in two normal subjects

ministration of the compound in one of the patients with haemochomatosis the reduced UIBC values increased, the other patient *showed no capacity for* binding serum iron throughout the entire experiment The *latter* patient was again *subjected to the investigation* after having been treated with desferrioximate for several months The results of the second investigation resemble as to values for transferrin bound iron total iron PPD oxidase and catalase activity *to the results obtained* in the first experiment made with this patient The *only difference* was observed in UIBC values 15 min after administration the reason being that the *partial depletion of iron from* the tissues caused by the long lasting treatment was registered in the second test with increased values of UIBC due *to transferrin freed from iron* As soon as 1 hour after the administration of desferrioxamine *B* in this *test UIBC values had returned to nought*

Discussion

The results of this study show that desferrioxamine B administered intravenously *in healthy persons* is well as in subjects with reduced or with in*creased iron levels and patients* with haemochromatosis binds immediately on application i e in 5 min the cir*culating transferrin* bound plasma iron

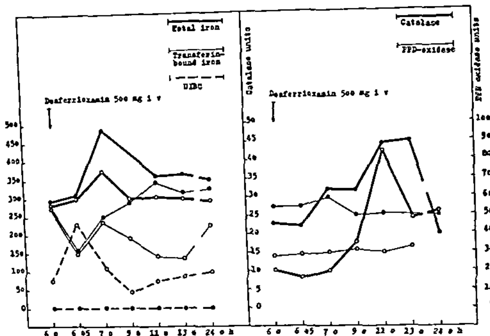


Table 4 Values of total serum iron transferrin bound iron unsaturated iron binding capacity and enzymatic activity of catalase and PPD oxidase in two patients with haemochromatosis *prior to and after application*

thus causing marked decrease of this iron Furthermore in the examined subjects whose initial iron levels before the administration of desferrioxamine B had been below normal values the determination of transferrin bound iron values 5 min after administration was nil This signifies that the desferrioxamine B compound in vivo in these cases directly binds all available plasma *iron before the compound can* diffuse in the tissues The results of UIBC determination reflect by raised values the condition of plasma transferrin freed from iron immediately on administration of desferrioxamine The amount of 500 mg of desferrioxamine B is known to be capable of binding about 45 mg of iron — it is hence understandable that a reduction — and in case of anemia even complete disappearance — of transferrin bound iron which becomes bound to desferrioxamine B is to be expected to take place in total plasma

In his earlier investigations Wohler observed that the total iron level in patients with *haemochromatosis rose* from 1 hour after application onwards (2) the same effect was registered in this study No increase of total iron after administration was noted in healthy subjects The values obtained in the course of the investigation in

determinations of copper and of PPD-oxidase activity show that the application produces no change

Conversely, in the majority of examined subjects, in two patients with haemochromatosis and in two healthy persons the values for enzymatic catalase activity show an increase 1, 3 and/or 5 hours after administration. The increase in catalase activity is not connected with the amount of circulating plasma iron since it was found in the normal subjects as well as in the other ones in whom no increase in total iron was registered and in those who had haemochromatosis when significant increase of total iron was registered. These data are complemented by the results of in vitro investigations in the activity of enzymatic catalase and PPD oxidase in serum loaded in vitro with the compound desferrioxamine B, ferrioxamine and with iron respectively. Enzymatic activity was found not to be changing in loaded serum in vitro when compared with the activity in not loaded serum

Although iron constitutes the prosthetic group of the peroxidative catalase enzyme these results on basis of experiments performed in vivo and in vitro allow for the conclusion that the increase of enzymatic activity of catalase in vivo after administration of the compound desferrioxamine B could not be brought into causative relation with the quantity of circulating plasma iron regardless of whether the iron was bound to transferrin or to desferrioxamine B. The increased enzymatic catalase activity observed in this study on administration of desferrioxamine B probably may be connected with the other accelerated oxidative metabolic processes described also by other authors in the course of treatment with the compound desferrioxamine B (12)

Conclusion

The concentration of transferrin bound iron, the iron binding capacity (UIBC), copper, as well as the enzymatic activity of catalase and PPD oxidase were studied prior to and after application of the compound desferrioxamine B (500 mg) in two patients suffering from haemochromatosis, in two normal controls and in 9 subjects with reduced, normal and increased serum iron levels respectively

The results showed that as early as 5 min after intravenous administration of the conpound there ensued marked reduction of transferrin bound iron with a concurrent sudden increase in UIBC. One hour after administration the values for transferrin bound iron and UIBC approximated those registered before loading to return to initial values after 24 hours. The application of the compound produced no effect upon the level of copper and the enzymatic activity of PPD oxidase in all examined subjects

It was observed in two patients with haemochromatosis that the level of total serum iron (transferrin bound iron with iron bound to desferrioxamine B) showed a sudden rise after the administration while the values for total iron ranged in physiological variations in the control test

Enzymatic catalase activity in 8 of

11 studied subjects and in both patients with haemochromatosis regardless of the quantity of iron in circulating plasma 1, 3 and/or 5 hours after administration of desferrioxamine B showed increased activity and consecutive reduction to initial values after 24 hours

The in vitro loading of the serum with the compound desferrioxamine B ferrioxamine and trivalent iron respectively indicates that the loading in vitro does not change the enzymatic activity of PPD oxidase and catalase

We are indebted to pharmacist Andrina Stojanovski and laboratory technician Josipa Franjkovic for technical collaboration

References

1 Bickel M Gaumann E Keller Schierlein, W Prelog V Vischer F Wettstein, A. and Zahner H Experientia 16 129 (1960)

2 Bisherige Erfahrungen mit Desferrioxamin bei pathologischen Eisenablagerungen CIBA (1962)

3 *Dille R S A. Watkins C H J Lab a* Clin Med *33* 480 (1948)

4 Heilmeyer L. and Wohler F *Dtsch Med* Wschr *87* 2661 (1962)

5 Heilmeyer L and Plotner K Das Serumeisen und die Eisenmangelkrankheit Jena (1937)

6 *Hoyer G Scand. J Clin. Lab Invest 2* 125 (1950)

7 Julien CL Lemonnier E Etienne G., Rubmann, A. and Caroli J Arch Malad Appar Dig et Nutr 52 669 (1963)

8. Keberle H and Fruh F Forschungslaboratorien CIBA Persohnliche Mitteilung

9 Keler Bačoka M and Hahn A Lij vjes (in press)

10 Keler Bačoka M and Hahn A Klin Wschr *39* 1070 (1961)

11 *Ventura S J Clin. Path 5 271 (1952)*

12 Wohler F Acta haematol *30* 65 (1963)

From the Department of Medicine (Head J Waldenström MD) University of Lund Allmänna sjukhuset Malmö Sweden

Simplified method for determining carbon monoxide hemoglobin saturation in diagnosis of hemolytic disorders

By KARL GYDELL

The value of carbon monoxide hemoglobin (COHb) determinations in the diagnosis of hemolytic states is well documented (12, 7, 4, 5, 2) An advantage of this test compared with the isotope methods (Cr^{51} and Fe^{59}) is that it can be used repeatedly *i e* for evaluating the effect of a given therapy The COHb method has been used mainly in research work concerning hemolytic problems It is not used so widely in clinical routine work There are probably several reasons for this restricted use of the test One of the most important is probably of technical nature *viz* the complicated methods for determining the very low concentrations of endogenous carbon monoxide The method that has been used longest is the thermochemical or "Hopcalite method" originally described by Sjöstrand (11) and later modified by Linderholm and Sjöstrand (8) The thermochemical method is however rather complicated and laborious For optimal function the system should preferably operate continuously The author used the method for two years and found it very accurate The methodological error was found to be about ±0 03 % COHb (6) There are also colorimetric methods available for determining carbon monoxide in low concentrations A very sensitive method is described by Shepherd (10) In that test a CO sensitive substance is enclosed in special glass tubes which are commercially available[1] Andersson and Dahlström (1) have described a method in which the colour change obtained when the tubes are exposed to a CO containing gas sample can be read photometrically The author has used this method with slight modifications in a large number of investigations and has found it reliable and handy (6) A disadvantage of this method is that every new set of indicator tubes must be calibrated which is a rather laborious and time consuming procedure

[1] In Sweden from LKB Produkter Fabriksaktiebolag Stockholm



Fig 1 The Drager suction pump connected to an indicator tube

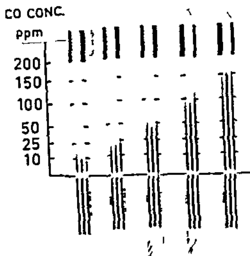


Fig 2 Propagation of the colour zone in 5 Drager test tubes exposed to carbon monoxide in concentration steps from 150 to 10 ppm

A simple and rather accurate variant of the colorimetric method for determining low CO concentrations is described below

Alveolar air samples from the subjects to be investigated were obtained by rebreathing pure oxygen in a rebreathing aggregate designed by AGA Lidingo (AGA MF 1500)

The CO concentrations in the rebreathed gas samples were measured with special indicator tubes (Type 5/b) manufactured by Dragerwerk Lubeck (3) These test tubes covers a measuring range from 5 to 200 ppm The gas sample is sucked through the tube with a pump (Fig 1) and a colour change is obtained in the indicator substance which contains a mixture of iodine pentoxide and sulphuric acid The gas volume needed for one analysis is one liter which can be sucked through the indicator tube in two minutes The final level of the colored zone is read against the scale of the tubes

Results and discussion

Fig 2 gives a reproduction of 5 test tubes exposed to CO mixtures containing from 150 to 10 ppm and illustrates the levels of the coloured zones The results of two double determinations are given in Fig 3 In order to compare the readings of the Drager CO detector tubes with the colorimetric method according to Andersson and Dahlstrom (1) estimations were made simultanously in 31 tests at various CO concentrations The results are given in Fig 4 The vertical lines in the diagram mark the range within which the various examiners considered the colour to cease The stan

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the blood can be calculated (3) By a slight modification of an ordinary spirometer for BMR investigations it may be possible to perform the re breathing procedure without any special apparatus

Summary

A simplified method for measuring carbon monoxide concentrations in gas samples after rebreathing pure oxygen with the use of Dräger CO detector tubes (Type 5/b) with a measuring range of 5 to 200 ppm is described The corresponding carbon monoxide hemoglobin concentration in the blood is calculated with Haldane s equation A comparison with another method for carbon monoxide determination indicates that the method described is accurate enough for detecting significant hyperhemolysis in clinical routine work

References

1 Andersson T and Dahlstrom H A clinical method for the determination of carbon monoxide in air Sience Tools The LKB Instrument J 5 9 1958

2 Coburn R F Williams W J and Forster R L Effect of erythrocyte destruction on carbon monoxide production in man J Clin Invest 43 1098 1964

3 Drager Heft Nr 54/1961 Information on the Drager Gas Detector

4 Engstedt L Endogenous formation of carbon monoxide in hemolytic disease Acta Med Scand 159 (suppl 332) 1957

5 Gydell, K Transient effect of nicotinic acid on bilirubin metabolism and formation of carbon monoxide Acta Med Scand 167 1 1960

6 Gydell K To be published

7 Hallberg L Blood volume hemolysis and regeneration of blood in pernicious anemia Studies based on the endogenous formation of carbon monoxide and determinations of the total amount of hemoglobin Scand J Clin. Lab Invest. 7 (suppl 16), 1955

8 Linderholm H and Sjostrand T Determination of carbon monoxide in small gas volumes Acta Physiol Scand 37 240 1956

9 Moeschlin S Klinik und Therapie der Vergiftungen Thieme Verlag Stuttgart 1964 (p 264)

10 Shepherd M Rapid determination of small amounts of carbon monoxide Anal. chem 19 77 1947

11 Sjostrand, T A method for the determination of carboxyhemoglobin concentrations by analysis of the alveolar air Acta Physiol Scand 16 201 1948

12 Sjostrand T Endogenous formation of carbon monoxide in man under normal and pathological conditions Scand J Clin Lab Invest. 1 201 1949

13 Wiklander O Blood volume determinations in surgical practice Acta Chir Scand. Suppl 208, 1956

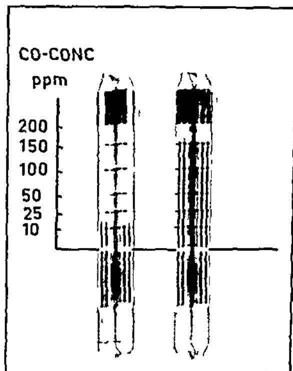


Fig 3 Two pairs of double determinations at different carbon monoxide concentration In the left pair there is a slight discrepancy between the positions of the colour levels No obvious difference can be seen in the right pair

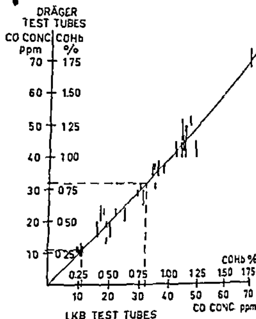


Fig 4 Relations between photometric CO determinations with "LKB" test tubes (abscissa) and direct reading from the same gas sample in Drager s test tubes (ordinate) Besides the CO concentrations the corresponding COHb values are given The interrupted lines denote the normal ranges of the endogenous CO and COHb values

dard deviation of these determinations was ±2 7 ppm Complete calculation of the COHb level in the blood from the CO concentration in alveolar air according to Haldane's equation requires estimation of the oxygen concentration in the gas samples (11 13) Provided that the rebreathing procedure is performed in a correct manner so that no leakage occurs in the connection between the patient and the aggregate the oxygen concentration after a rebreathing time of 15 minutes in pure oxygen varies but little from 95 volume per cent Introduction of this value as a constant in Haldane s equation gives a factor of 0 025 which has been used here for converting a given CO concentration in ppm to COHb in per cent saturation (COHb per cent$=CO_{ppm}\times 0\,025$) The aforementioned reading error of ±2 7 ppm thus corresponds to 0 07 per cent COHb This error is much larger than that of the two other methods mentioned above but the accuracy of the Dräger CO detector tubes seems to be sufficient for discovering hyperhemolysis in routine clinical work These tubes can also be used for direct analysis of the CO concentration in *expired* air in suspected CO intoxication (9) From the CO concentration obtained a corresponding COHb level in

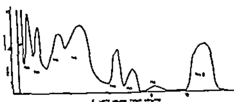


Fig 1 Radioactivity curve from a partial hydrolysate of red cell ghosts separated on a Dowex 50 (8% DVB) column Incubation time 16 hrs in 18 C



Fig 2 Radioactivity curve (the continuous line) of the material from peak No 5 Fig 1 separated on a Dowex 1 (2% DVB) column The broken line represents formic acid concentration

Only UV absorbing material was found in subfraction 6 (fig 2) The material from peak 8 ("phosphoryl choline") could also be separated into 6 fractions by Dowex 1 column chromatography Phosphopeptides were found in subfraction 1 and 4 Number 2 and 3 contained phosphorylcholine and 5 and 6 both contained UV absorbing material (fig 3)

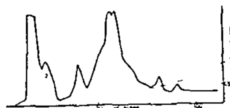


Fig 3 Radioactivity curve of the material from peak No 8 Fig 1 See legend for Fig 2

The UV absorbing material was further purified by high voltage electrophoresis using Whatman No 3 paper The electropherograms were run in the Wieland Pfleiderer apparatus at 2650 V and 55 mA for 60 min at 4—6 C using a 0 05 M citric acid sodium citrate buffer of pH 3 6 (1 3) The UV absorbing spots were then eluted from the electropherograms with water Samples from the eluate were analyzed for ribose (10) and phosphorus (11) The remaining material in amounts not exceeding a UV absorbance value of 1 0 cm at 260 mμ was all hydrolyzed in 0 5 ml of concentrated formic acid for 30 min in sealed tubes in 175 C The hydrolysates were analyzed by thin layer chromatography together with the reference substances adenine guanine uracil methylcytosine and cytosine as described by Josefsson (12) with slight modifications In some experiments the eluted material was concentrated *in vacuo* and then dissolved in 0 01 N HCl and the UV spectrum was measured in a Perkin Elmer Model 137 UV spectrophotometer The UV spectrum at pH 2 0 for the substances are illustrated in fig 4 and 5

In thin layer chromatography the UV absorbing material from the electropherograms in all cases ran parallel with the reference cytosine In addition the UV spectrum of all substances was in good agreement with that of cytidine (13) Attempts were made to estimate ribose and phosphorus in the eluted UV absorbing spots but the values obtained were

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The Occurrence of a Metabolically Active Cytosine compound in a Protein Fraction from Human Erythrocyte Ghosts

By GUNNAR RONQUIST and GUNNAR ÅGREN

The uptake of labeled inorganic phosphate ($^{32}P_i$) by human erythrocyte ghosts has previously been studied (1, 2) It was also shown that ^{32}P was incorporated into phosphopeptides as well as into phosphorylethanolamine and phosphorylcholine This process was rather slow and the labeling of the phosphopeptides preceeded that of phosphorylethanolamine and phosphorylcholine

It has also been reported (3, 4) that whole human erythrocytes incorporate $^{32}P_i$ mainly into phosphatidic acid Rowe (5) in studying the metabolism of unfractionated blood cells observed a more uniform $^{32}P_i$ labeling of the cellular phospholipids but it has been claimed (6), that these results likely are not to be attributed to the erythrocytes Thus James et al (7) Marks et al (8) and Buchanan (9) all claim that any synthesis of lipid in blood is a result of white cell and reticulocyte activity

Some data are now reported as evidence for a biosynthesis of phospholipids in the red cell membrane itself

Ghosts were prepared from human erythrocytes which had been separated from most of the white cells (2) They were incubated for various periods with $^{32}P_i$ and then precipitated with trichloroacetic acid (1, 2) The precipitate was dried with ethanol and the lipids were removed by extraction twice with ethanol/ether at 45° C followed by ether

The residual "protein" fraction was partially hydrolysed in 2 N HCl for 20 hrs and the hydrolysate was applied to a Dowex 50 column and eluted with 12 column volumes of 0 01 N HCl The elution pattern is shown in fig 1* All peaks contained labeled phosphopeptides but peak 5 and 8 contained in addition labeled phosphorylethanolamine and phosphorylcholine respectively

All these peaks could be further separated into several subfractions on a Dowex 1 formate column with gradient elution 0 → 1 M formic acid Thus peak 5 ("phosphorylethanolamine") was further separated into 6 subfractions of which 1 and 2 contained phosphopeptides Phosphorylethanolamine was found in 3 and 4

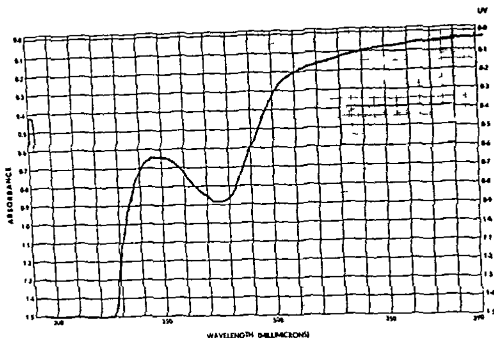


Fig 5 UV spectrum at pH 2 0 of the eluated UV absorbing spot in the electropherogram of fraction 6 Fig 2

ATP necessary for a formation of phosphorylethanolamine and phosphorylcholine is also formed in the red cell membrane (16 17)

References

1 Ågren G Hallberg B and Ronquist G Acta Chem Scand 16 17 0 (1962)

2 Ågren G and Ronquist G Acta Chem Scand 18 2029 (1964)

3 Lassant Diament M and Polonovski J Bull Soc Chim Biol 42 337 (1960)

4 de Gier J Mulder E Mulder I and van Deenen L L M Koninkl Ned Akad Wetensch Proc Ser B 64 274 (1961)

5 Rowe C J Biochem J 3 438 (1955)

6 L L M van Deenen and J de Gier in Charles Bish p s The Red Blood Cell p 243 Academic Press New York 1964 James A T Lovelock J E and Webb J I W Biochem J 3 106 (1959)

8 Marks P A Gellhorn A and Kidson C J Biol Chem 235 2579 (1960)

9 Buchanan A A Biochem J 75 315 (1960)

10 Mejbaum W Z Physiol Chemie 258 117 (1939)

11 Weil Malherbe H and Green R H Biochem J 49 286 (1951)

12 Josefsson L Biochem Biophys Acta 72 133 (1963)

13 Pabst Laboratories Division of Pabst Brewing Company 1037 W Mc Kinley Ave Milwaukee 5 Wisconsin cirkular 01 —10 Jan., 1956

14 Kennedy E P and Weiss S B *J Am Chem Soc.* 77 250 (1955)

15 Kennedy E P and Weiss S B J Biol Chem 222 193 (1956)

16 Ronquist G and Ågren G Nature 205 1021 (1965)

17 Ronquist G and Ågren G Formation of Adenosine Triphosphate by Human Erythrocyte Ghosts To be published

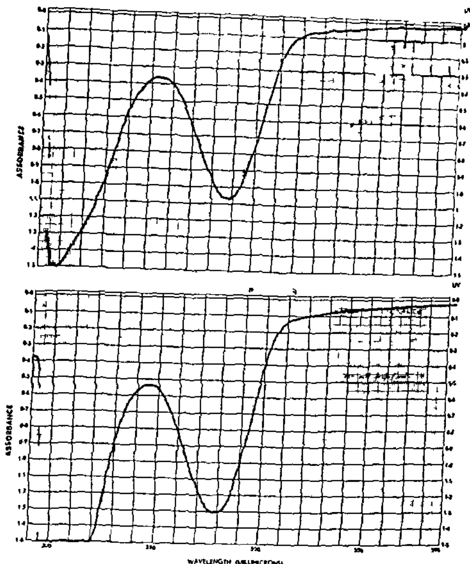


Fig 4 UV spectra at pH 2 0 of the eluated UV absorbing spots in the electro pherograms of fraction 5 (upper) and fraction 6 (lower) Fig 3

rather low and too fluctuating to give reliable information

It seems that the found cytosine compound is rather hardly bound to the stromal proteins and since cyto sine was isolated as a compound la beled with $^{32}P_i$ it seems probable that this compound might be cytidylic acid We have previously reported the isolation of comparatively large amounts of phosphorylethanolamine and phosphorylcholine from the same source (*2*) It is therefore suggested that the isolated cytosine compound together with the latter compounds are involved in the synthesis of phos phatidylethanolamine lecithin and sphingomyelin in the red cell mem brane in the same pathway as describ ed by Kennedy and Weiss (14 15) for other tissues

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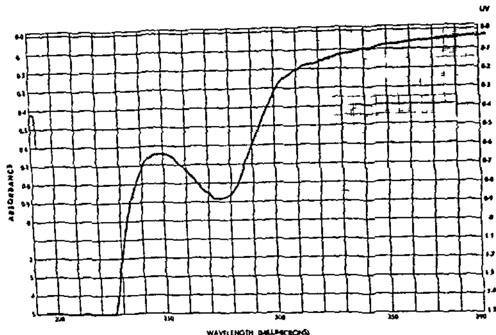


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References

1 Ågren G Hallberg B and Ronquist G Acta Chem Scand 16 1 0 1962)
2 Ågren G and Ronquist G Acta Chem Scand *18* 2029 1964)
3 La sant D an ent M and Polonovski J Bul Soc Chim Biol 42 33 1960
4 de Geer J Mulder L Mulder E and van Deenen L L M Kon nkl Ned Akad Wetens Proc Ser B 64 2 4 1961
5 Bowe J *Biochem J* 3 438 1953
6 L L M an Deenen and J de Geer in Charles Bishop s The Red Blood Cell p 41 Academic Press New York 1964 James A T Lovelock J L and Webb J L W Biochem J 3 106 19 9
8 Marks P A Gellhorn A and Kidson C J Biol Chem 235 2a 9 (1960)
9 Buchanan A A Biochem J 75 315 (1960)
10 Mejbaum W Z Physiol Chemie 258 117 (1939)
11 Weil Malherbe H and Green R. H Biochem J 49 286 (1951
12 Josefsson L Biochem Biophys Acta 2 133 (1963)
13 Pabst Laboratories Division of Pabst Brewing Company 103 W Mc Kinley Ave Milwaukee 3 Wisconsin Circular 01 —10 Jan 1956
14 Kennedy E P and Weiss S B J Am Chem Soc 7 2a0 (1955)
15 Kennedy E. P and Weiss S B J Biol Chem 222 193 (1956
16 Ronquist G and Ågren G Nature 205 1021 1965)
17 Ronquist G and Ågren G Formation of Adenosine Triphosphate by Human Erythrocyte Ghosts To be published

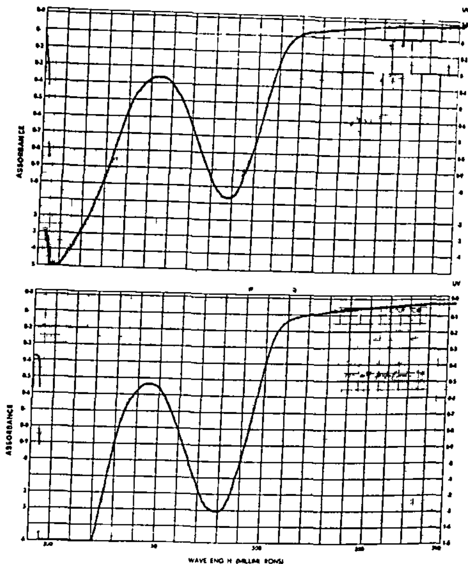


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établirent la notion d'agranulocytose périphérique insistaient sur l'aspect normal du myélogramme. Normal et même hyperplasique initialement au point que cette hyperplasie granulocytaire était comparée à l'érythroblastose médullaire des anémies hémolytiques. Les images d'hypoplasie granulocytaire étaient tenues pour rares et toujours tardives (17). Les études récentes (1) *montrent qu'au moins dans* la grande majorité des cas l'évolution des myélogrammes est toute différente et même exactement contraire : 1) l'atteinte du tissu granulopoïétique médullaire est extrêmement fréquente sinon constante au stade initial ; 2) ces lésions médullaires sont variées mais le désordre le plus commun est le blocage : l'arrêt de maturation au stade promyélocytaire ; 3) ces lésions sont *éphémères et très rapidement corrigées*. C'est alors mais alors seulement *que l'on note l'hyperplasie granulocytaire*.

Ainsi ces *images médullaires des agranulocytoses du pyramidon* sont assez originales et doivent être distinguées aussi bien des images normales décrites peut-être par erreur en 1933 que des lésions médullaires des aplasies dues aux antimitotiques qui ne sont jamais ou presque jamais aussi vite réparées.

La chronologie véritable ne permet pas de retenir les explications des lésions médullaires *qu'on donnait* naguère ni l'atteinte tardive des granulocytes médullaires *par la substance nocive* ni l'épuisement médullaire secondaire. Elle s'accorderait bien avec l'hypothèse d'une altération précoce du tissu granulopoïétique médullaire. Il est difficile, en l'état actuel de discerner si les lésions granulocytaires initiales sont *purement médullaires ou* si ces lésions sont à la fois médullaires et périphériques ou encore si une lésion initialement périphérique est suivie très rapidement par un mécanisme qu'il faudrait préciser d'un arrêt de la maturation médullaire. On doit en tout cas tenir compte de la précocité et de l'allure de ces lésions médullaires *dans la discussion du mécanisme* de l'agranulocytose du pyramidon. On doit en tenir compte aussi dans la discussion du diagnostic. Ces moelles promyélocytaires sont parfois fort émouvantes : le diagnostic de leucémie aiguë à promyélocytes peut être évoqué. Les promyélocytes de l'agranulocytose du pyramidon sont en *règle générale normaux et très différents* des promyélocytes leucémiques *altérés avec leurs grosses granulations*. Les difficultés n'en sont pas moins *parfois très grandes. Les meilleurs cytologistes* ont pu s'y tromper et c'est avec bonheur que l'on voit la maturation granulocytaire survenant, les myélogrammes ultérieurs démentir le cruel diagnostic initial.

L'importance de la plasmocytose médullaire peut être responsable d'autres difficultés de diagnostic. La plasmocytose *médullaire fréquente* mais habituellement modérée et isolée *peut parfois être forte*, être accompagnée par une plasmocytose sanguine, par de franches modifications des protéines sériques. À côté de la plasmocytose une prolifération de cellules réticulaires plus ou moins orien

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Remarques sur l'Agranulocytose du Pyramidon

Par JEAN BERNARD

Une part importante de l'oeuvre du Professeur Jan Waldenstrom est consacree a l'etude de l'action des medicaments sur le sang. C'est a ce propos que nous apportons ici en hommage a Jan Waldenstrom ces quelques remarques sur l'agranulocytose du pyramidon. Ces remarques sont fondees sur l'examen des cas suivis dans notre Institut a Paris de 1955 a 1965.

Vers 1952 la description de l'agranulocytose du pyramidon paraissait definitive comme semblait simple et assuree l'explication qu'on proposait de son mecanisme. Les experiences d'Ackroyd ou plutot l'interpretation qui en decoulait avaient ete transposees des plaquettes et du sedormid aux leucocytes et au pyramidon (bien que l'agglutination des leucocytes n'etait observee qu'avec le serum preleve en pleine crise et que dans aucun cas on n'ait pu jusqu'a un cas recent (15) restaurer le pouvoir agglutinant d'un serum preleve ulterieurement en lui ajoutant du pyramidon (18) alors que c'est regulierement le cas par exemple dans les purpuras au sedormid). L'agranulocytose du pyramidon pense t on alors est une agranulocytose peripherique liee a l'atteinte des elements granuleux dans le sang circulant, elle se separe par la des agranulocytoses centrales medullaires, provoquees par les medicaments myelotoxiques comme les moutardes, le benzene le chloramphenicol. Elle est pure purement leucocytaire. Les globules rouges et les plaquettes restent normaux alors qu'ils sont presque toujours alteres dans l'agranulocytose centrale simple symptome d'une aplasie medullaire globale. Elle est expliquee par l'hypothese immuno allergique alors que les aplasies medullaires medicamenteuses, celles par exemple du chloramphenicol demeurent souvent obscures. Elle peut etre guerie par un traitement vigoureux et urgent. Elle devrait aisement etre prevenue grace a la connaissance de sa cause et de sa physio pathologie. Cette agranulocytose du pyramidon qu'on se represente ainsi en 1952 comme une agranulocytose peripherique pure simple curable aisement prevenue qu'est elle devenue en 1965?

Les descriptions de 1952—1955 qui

conditions étiologiques l'induction des accidents par une toute petite dose s'accordent très bien avec l'hypothèse immuno-allergique. On est d'abord tenté d'accuser l'insuffisance des techniques (mais elles se sont améliorées quoique bien des techniques sérologiques n'aient pas encore été mises en oeuvre) ou le retard de l'examen (mais il est précoce avec la dose test). Mais peut-être les difficultés tiennent-elles au fait que l'on n'étudie ni les substances vraiment responsables ni la cellule vraiment touchée. Ce n'est peut-être pas le pyramidon entier mais plutôt tel ou tel de ses catabolites qui induit la réaction: les recherches entreprises dans cette direction pourraient être développées. Ce n'est peut-être pas le polynucléaire neutrophile mûr mais plutôt l'un de ses précurseurs qui est atteint le premier. Les données cliniques et cytologiques, la lésion précoce de la moelle granulopoïétique, l'altération légère des autres séries pourraient s'accorder avec cette explication. Les méthodes immunologiques qui ont rendu tant de services dans l'étude des globules du sang ne sont que malaisément applicables aux cellules médullaires. D'autres techniques sont à l'étude. Améliorées, elles permettront de vérifier ou d'infirmer cette hypothèse de travail.

Le pyramidon peut-il, outre l'agranulocytose aiguë, provoquer des cytopénies chroniques? Il n'est pas rare dans l'anamnèse des malades avec cytopénies chroniques de trouver des prises répétées de pyramidon. Il est difficile d'affirmer leur intervention et le cas échéant de préciser le mode: 1) action directe du pyramidon sur les cellules granulocytaires; 2) mécanisme allergique, la leucopénie étant entretenue par des absorptions fréquentes du médicament contre lequel s'est développé un anticorps; 3) formation d'autoanticorps. Dans une observation d'anémie hémolytique au pyramidon (3) le sérum malade est capable d'agglutiner des hématies normales ayant subi un *contact prolongé* avec le médicament puis trypsinisées *et tout se passe comme si le pyramidon altérait l'hématie la rendant antigénique pour elle-même*. Quelques constatations sérologiques *ont permis récemment d'envisager un* mécanisme analogue pour certaines leucopénies chroniques (8). Chez certains malades dont le passé pathologique chargé a entraîné un long usage médicamenteux (névropathes, rhumatisants) ou après une intoxication médicamenteuse intense on constate parfois à l'aide des tests direct et indirect de consommation de l'antiglobuline l'apparition de substances gamma-globuliniques ayant une spécificité antileucocytaire. L'altération médicamenteuse est peut-être capable de provoquer l'apparition parfois transitoire d'auto-anticorps.

La découverte du mécanisme immuno-allergique des cytopénies médicamenteuses a donné bonne conscience et confort intellectuel aux hématologistes. Conscience *et confort* peut-être prématurés. Car de très importants problèmes ne sont pas résolus. Des dizaines de milliers de personnes en France seulement consom-

tees vers le plasmocyte peut etre trouvee, des adenopathies infiltrees de cellules reticulaires et de plasmocytes ont ete a titre exceptionnel observees (4, 5) Entre agranulocytose medicamenteuse et sarcomatose, le diagnostic peut etre tres malaise Certes l'etude cytologique attentive permet le plus souvent de poser le diagnostic exact que l evolution confirme Jusqu a present, l'avenir de ces malades a ete bon L'hypothese qui admet des liens entre agranulocytose et myelome n'a pas ete confirmee par les faits (11) On comprend a la lecture de certains documents qu'elle ait ete proposee

On crut d abord que les lignees erythrocytaires et plaquettaires etaient absolument intactes que la lignee granulocytaire etait seule touchee En fait, l'etude des observations met en evidence une atteinte des autres series rouge et plaquettaire Cette atteinte est legere, passagere, reconnue seulement par une analyse attentive (1) La duree de vie des divers elements du sang peut expliquer que cette atteinte soit parfois meconnue La nature des troubles, en particulier la reticulocytopenie temoignent en faveur d une alteration des tissus erythropoietiques et thrombopoietiques medullaires L interpretation de ces troubles est malaisee et on ne sait s il faut les lier a une action directe de la substance nocive sur la moelle (et sur quel element medullaire?) ou envisager d autres hypotheses

Les retouches ainsi apportees aux premieres descriptions permettent de tracer un tableau qui a une certaine valeur diagnostique Une agranulocytose profonde, accompagnee d une tres discrete insuffisance rouge et plaquettaire, d'un arret passager de la maturation granulocytaire, surtout d un arret promyelocytaire, evoque l empoisonnement par le pyramidon

Quel est le mecanisme exact de l'agranulocytose du pyramidon? Le leucocyte participe t il activement a la constitution de l antigene formant avec le pyramidon un complexe qui devient antigenique ou ce leucocyte n'est il atteint que passivement par un conflit immunologique qui se deroule a sa surface? (16, 19)

Depuis dix ans les immunologistes discutent sans s'accorder et, comme au temps de la logique formelle, alignent en face de chaque hypothese les arguments "pro et contra Les controverses sont brillantes, elles seraient cependant plus fondees si l anticorps qui est leur objet etait habituellement mis en evidence Il n'en est rien et dans la grande majorite des observations recentes il n'a pas ete possible de demontrer la presence de cet anticorps Dans un seul cas, il a ete possible de demontrer un anticorps actif seulement en presence du pyramidon (15) Cet echec habituel a souvent ete note aussi par les medecins exerçant dans les pays dont la morale permet (ouvertement ou hypocritement) par l administration de dose test de mettre deliberement en danger la vie de leur prochain, en provoquant une nouvelle agranulocytose Ces echecs sont d autant plus surprenants que l'allure clinique de l agranulocytose du pyramidon, sa violence et sa regression ses

conditions etiologiques l induction des accidents par une toute petite dose s accordent tres bien avec l hypothese immuno allergique On est d abord tente d accuser l insuffisance des techniques (mais elles se sont ameliorees quoique bien des techniques serologiques n aient pas encore ete mises en oeuvre) ou le retard de l examen (mais il est precoce avec la dose test) Mais peut etre les difficultes tiennent elles au fait que l on n etudie ni les substances vraiment responsables ni la cellule vraiment touchee Ce n est peut etre pas le pyramidon entier mais plutot tel ou tel de ses catabolites qui induit la reaction les recherches entreprises dans cette direction pourraient etre developpees Ce n est peut etre pas le polynucleaire neutrophile mur mais plutot l un de ses precurseurs qui est atteint le premier Les donnees cliniques et cytologiques la lesion precoce de la moelle granulopoietique l alteration legere des autres series pourraient s accorder avec cette explication Les methodes immunologiques qui ont rendu tant de services dans l etude des globules du sang ne sont que malaisement applicables aux cellules medullaires D autres techniques sont a l etude Ameliorees elles permettront de verifier ou d infirmer cette hypothese de travail

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La decouverte du mecanisme immuno allergique des cytopenies medicamenteuses a donné bonne conscience et confort intellectuel aux hematologistes Conscience et confort peut etre prematures Car de tres importants problemes ne sont pas resolus Des dizaines de milliers de personnes en France seulement consom

ment chaque annee du pyramidon ou l'une des cent specialites en contenant Quelques unes seulement seront victimes de la drogue Les victimes memes ne sont pas egales victimes L'immunisation a un même medicament varie d'un individu a un autre Ces variations peuvent porter sur le type serologique d anticorps, sur sa specificite Le pyramidon peut provoquer tantot l apparition d'un puissant anticorps de type reaginique entrainant des accidents cutanes sans leucopenie (12) tantot l'apparition d'un anticorps leucopeniant agglutinant les leucocytes en presence de pyramidon (15) La specificite des anticorps allergiques cytopeniants varie d un malade a un autre Tel produit provoquera selon le malade, une anemie hemolytique, un purpura thrombopenique ou une leucopenie Cette diversite l inegale sensibilite des etres humains au pyramidon seraient bien expliquees par des facteurs hereditaires, par une predisposition constitutionnelle Jusqu a present aucun fait positif n a ete apporte qui temoigne en faveur de cette hypothese Mais ces etudes pharmacogenetiques n ont ete qu ebauchees Il serait important de chercher dans les familles des malades des sensibilisations latentes dans le cas de contact familial avec un produit De telles sensibilisations latentes decouvertes par un examen fortuit ont ete recemment signalees (14) Il est utile de rappeler certaines constatations experimentales montrant que la capacite de sensibilisation a un meme antigene artificiel est transmise hereditairement (13) Il est difficile de prevoir la nature de ce desordre hereditaire predisposant anomalie dans le catabolisme meme du medicament, anomalie de la constitution des proteines sur lesquelles le medicament ou sa metabolite pourra se fixer

Ainsi en l etat actuel, la prevention de l'agranulocytose du pyramidon ne peut se fonder, comme celle des anemies hemolytiques enzymoprives sur le depistage biochimique des individus fragiles Cette prevention repose sur la limitation de l emploi du pyramidon Limitation generale et interdiction definitive aux individus victimes une fois de la drogue Cette interdiction ne peut etre fondee que sur un diagnostic assure Les medecins avertis savent toute l importance d une enquete minutieuse, patiente, repetee, prolongee qui finit par arracher au malade le souvenir de comprimes de suppositoires oublies ou negliges Mais la diversite des formes medicamenteuses et de leurs appellations entrave souvent l enquete et en compromet le resultat Comment le medecin le plus erudit peut il connaitre cette liste mouvante qui on l a dit plus haut comprend plus de cent noms pour le seul pyramidon Un dictionnaire est necessaire (2) qui fournit le nom de tous les coupables et vient utilement orienter l interrogatoire et la prevention L effort efficace des organismes internationaux ordonne la nomenclature mais rien ne limite la multiplication des preparations commerciales Ce desordre entraine l usage l abus presque inconscient d un medicament parfois dangereux Une double limitation semantique et pratique reduisant le vo

cabulaire et réduisant l'emploi de la drogue est bien souhaitable

Bibliographie

1 Bernard (Jean) Najean (Y) et Binet (J L) L'agranulocytose provoquée par les dérivés de l'amidopyrine Etude de 7 observations (Bull et Mem Soc Med Hop Paris 1960 76 1134)

2 Bernard (Jean) Dausset (J) et Magis (C) Anémies leucopénies thrombopénies provoquées par *les medicaments* (I vol 1965 Masson et Cie éd)

3 Bernasconi (C) Bedarina (G) Pollini (G) et Sartori (S) Studio del mecanismo di emolisi in un caso di anemia emolitica acquisita di piramidone (Haematologica 1961 *48* 697)

4 Bierme (R et S) Colombies (P) et Bernard (Jean) Agranulocytose due a une phenothiazine (Soc Fr d'Hemat 15/6/1964 in Nouv Rev Franç d'Hémat sous presse)

5 Bousser (J) Agranulocytose curable accompagnée de plasmocytose et d'hyperglobulinemie transitoire (Sang 1957 *28* 364)

6 Dausset (J) Agranulocytoses et leucopenies *immunologiques Aspects sérologiques* (Sang 1954 25 683)

Dausset (J) et Bergerot Blondel (Y) Étude d'un anticorps allergique actif en présence de para amino salicylate de soude (P A S) contre les hématies les leucocytes et les plaquettes humaines (Vox Sang 1961 *6* 91)

8 Dausset (J) et Contu (L) Quelques remarques sur les cytopenies medicamenteuses immunologiques (Nouv Rev Franç Hémat 1964 4 21)

9 Dausset (J) et Thierfelder (S) Spezifitatsunterschiede *bei allergischen* anti P A S antikorpern (Klin WOsch 1964 42 272)

10 Dreyfus (B) Cytopenies medicamenteuses d'origine allergique (Presse Med 1959 67 1464)

11 De Gennes (L) et Marchal (G) *La plasmocytose* et l'hyperglobulinemie dans les syndromes agranulocytaires Les plasmocytes allergiques (Presse Med 1954 *6*? 965)

12 Halpern (B N) The immunological character of humoral antibodies in drug allergy (in Sensitivity reactions to drugs A symposium Oxford 1958, Blackwell ed)

13 Levine (B B) Ojeda (A) et Benacerraf (B) Basis for the antigenicity of hapten poly L Lysine conjugate in random bred guinea pig (Nature 1963 *200* 544)

14 Magis (C) Contu (L) et Dausset (J) Mise en évidence de deux anticorps antiplaquettaires allergiques d'origine medi*camenteuse actifs l'un en présence de* trimeprazine l'autre en présence de bromphéniramine (IX° Congr Soc Europ Hémat Lisbonne 1963)

15 Magis (C) Thierfelder (S) Saint Paul (M) et Dausset (J) Etude sérologique d'une agranulocytose allergique à l'amidopyrine (Nouv Rev Franç Hemat 1962 2 602)

16 Miescher (P) Leucopénies et agranulocytoses d'origine allergique (Sang 1955 26 71)

17 Moeschlin (S) Granulocytopenie immu*nologique et agranulocytose Aspects cli*niques (Sang 1955 *26* 32)

18 Moeschlin (S) et Wagner (K) Agranulocytosis due to the occurence of leukocyte agglutinins (pyramidon and cold agglutinins) (Acta Haemat 1952 *8* 29)

19 Shulman (N R) et Rall (J E) Mechanism of blood cell destruction in individuals sensitized to foreign antigens (*Trans Ass Am Phys* 1963 *76* 72)

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Transient neutrophil agranulocytosis in a newborn with leucocyte antibodies of type anti-8a in the mother

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Transient neonatal granulocytopenia was described in 1950 by Slobody et al. (26) and in 1951 by Lehndorff (16). This condition is characterised by granulocytopenia in the newborn period usually appearing a few days after birth and generally remitting within the course of one to two months. Infections of various degrees may be found during this period but in many cases there is a complete lack of clinical symptoms. In the first publication by Slobody et al. the possibility of an isoimmunisation as the cause of the granulocytopenia was suggested and later positive evidence for such a mechanism was obtained.

In the literature several cases with neonatal granulocytopenia have been described where leucocyte antibodies were indicated as a possible cause (1, 2, 7, 8, 10, 15, 17, 24, 27). The syndrome has been reviewed e.g. by van Rood (21) and Dausset (5).

In healthy subjects leucocyte isoantibodies are only found as an effect of transfusion or pregnancy. The frequency of mothers with leucocyte antibodies has been found to increase with the number of pregnancies (18, 19, 22). This indicates that the foetus, like in Rh immunisation, can immunise the mother. A difference from Rh immunisation is, however, that these leucocyte antibodies, according to investigations by Jensen (11, 12) and Payne (18, 20), seem to produce leucopenia in the child only exceptionally. None of these authors could ascertain leucopenia even in cases with high antibody titres. Transplacental passage of antibodies was shown by Payne in one third of the cases. The number of leucocytes in this group of children did not differ from the number of leucocytes in a control group where the mothers lacked leucocyte antibodies.

Although cases have been described in which a relationship between granulocytopenia in the child and the presence of leucocyte antibodies in the mother seems very likely, there is nothing to prove that such a relation is a common phenomenon. Obviously several factors e.g. concerning properties of the antigens or antibodies pro-

duction of leucocytes in the infant etc may be involved The case presented below deserves interest because the leucocyte antibodies present were established to be group specific and consisting of IgG globulin

Case report

Boy born 8 11 1964 The mother was a 29 year old primi para blood group O Rh(+) earlier healthy no blood transfusions During the last trimestre minimal oedema and a slightly raised blood pressure occurred Delivery seven weeks before term. Birth weight 2 360 g Half an hour after delivery the child became cyanotic and showed signs of irregular breathing and was therefore admitted to the Pediatric Department. After 15 minutes in an environment of increased O_2 he was again in good condition Blood examination 12 hours after birth showed absence of the neutrophile peak present in normal newborns (total leucocytes 10 900 per mm³ of which 3 100 polynuclear cells per mm³) However the finding was not so striking that further study was considered necessary On the fourth day the serum bilirubin value was found to be 24 8 mg per 100 ml

The blood group of the child was O Rh(+) and the direct antiglobulin (Coombs) test was negative No irregular erythrocyte antibodies were found in the mother s serum An exchange transfusion was performed and again repeated two days later when the bilirubin concentration was 26 8 mg per 100 ml serum The bilirubin values after that showed a downward slope The patient s general condition was good and he gained weight steadily On the sixteenth day the boy became apathetic and cried faintly Neurological examination did not reveal any gross abnormalities The spinal fluid was faintly yellow with a total protein value of 195 mg per 100 ml cells 184 mononuclear 6 polynuclear and 25 erythrocytes per 3 2 mm³ Bacterial cultures from spinal fluid and blood were negative Penicillin (Doktacillin) was given as a prophylaxis A few days later the patient seemed to be free from symptoms A blood examination on the eighteenth day revealed a neutrophil agranulocytosis The total number of leucocytes was 7 200 per mm³ non segmented granulocytes 1 0 % polynuclear cells 0 % eosinophils 11 0 % lymphocytes 77 0 % monocytes 10.5 % and plasma cells 0 5 % Thrombocytes numbered 260 000 per mm³ A bone marrow specimen taken on December 1st showed a picture of agranulocytosis with almost complete "arrest" on the myelocyte stage The erythrocyte precursors were somewhat sparse but otherwise normal Megakaryocytes were few in number

The child s condition remained quite satisfactory there were no clinical signs of infection and he showed good increase in weight The blood picture was improved (Fig 1) Yet even after seven months there was a granulocytopenia (leucocytes 11 600 per mm³ segmented granulocytes 15 0 % lymphocytes 80 5 % and monocytes 4 5 %)

Serological examination

Serum samples from the mother were taken on December 8th i e four weeks after delivery Using the agglutination technique according to Dausset (4) leucocyte antibodies active against leucocytes from the father from the child and from twelve out of 30 O Rh(+) blood donors chosen at random were found in the serum The father belonged to blood group B Rh(+) In order to test the mother s serum for antibodies to the father s leucocytes the mother s serum was first absorbed with erythrocytes from a B person whose leucocytes gave a negative reaction The mother s antibody titre against the father s leucocytes was 1 8 against the child s 1 32 against three of the blood donors 1 8—32

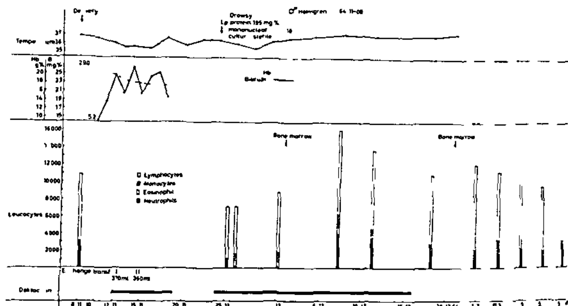


Fig 1 Course of the disease including blood examination from birth until twelve months of age

and against nine of the blood donors 1 2—8 Tests from the remaining eighteen blood donors gave negative reactions The examination was performed with defibrinated leucocytes

The results indicated the possibility of a relatively simple group specificity and therefore the serum was sent to the WHO Reference Laboratory for leucocyte grouping in Leiden for further study (Director J J van Rood M D) With a panel consisting of nine 8a positive and six 8a negative leucocyte samples it appeared that the antibodies in the serum recognized an antigen which showed a significant correlation with the antigen 8a (23) It was concluded that in all probability the serum had anti 8a specificity

Immunoglobulin examination

Serum was fractionated by gel filtration on a Sephadex G 200 column according to an earlier described technique (13) The fractions were concentrated and tested partly with the agglutination technique and partly with the antiglobulin consumption technique (9) As is shown in Fig 2 antibody activity was obtained only in the fractions corresponding to the

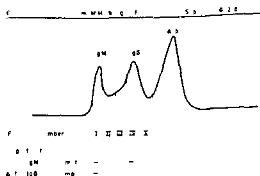


Fig 2 Fractionation and antiglobulin consumption experiment showing that the leucocyte antibodies of serum from the mother tested with leucocytes from the father consisted of IgG globulin

second main peak. This contains the main part of IgG and part of the IgA globulin in serum. The antiglobulin consumption test with a system specific to IgG globulin gave a positive reaction. The presence of antibodies of IgG type was therefore established but no antibodies of IgM type were demonstrated.

Discussion

The child described above was prematurely born. According to Jensen (11) there is an increased frequency of premature births in mothers with leucocyte antibodies. Increasing jaundice during the first few days of life necessitated two exchange transfusions. However, no red cell antibodies were demonstrated and no ABO incompatibility between mother and child was present. The experience from exchange blood transfusions in cases of Rh erythroblastosis indicates that the exchanges as such do not give granulocytopenia.

The fact that maternal leucocyte antibodies relatively often appear during pregnancy as the result of iso-immunisation, whereas leucopenia is an unusual phenomenon in the offspring, necessitates caution in any conclusions of a true causal relationship between the two findings. However, in the case here described there might be some basis for such a relationship since the antibodies were of IgG type giving a strong reaction with the child's leucocytes. It is well known that the IgG globulin passes the placenta. There might be several plausible explanations why granulocytopenia in the infant is rare in spite of maternal leucocyte antibodies.

1. Probably the amount of leucocyte antibodies and their physicochemical characteristics are of importance (9). However, the technique for leucocyte agglutination presently available is not quite ideal for quantitative determinations (5, 6). Therefore it may be difficult to draw valid conclusions from the antibody titres.

2. The antigen against which the antibodies are directed may be present in a number of cells in the organism which can contribute to a quick absorption and breaking down of the antibodies. Thereby the amount of antibodies bound to the leucocytes can be so low that the cells escape damage. Certain antigens are common to granulocytes, lymphocytes, thrombocytes and probably also to other cell types in the organism. Other antigens seem to be more specifically localised to a certain cell type (6). It might therefore be possible that a leucopenia could be developed by antibodies directed against antigens which exist almost exclusively in granulocytes but which are absent in most other cell types. Lalezari has recently presented evidence that the leucocyte antibodies active in his cases were pure anti-granulocyte antibodies (14). In our case the antibodies had the specificity anti-8a. At the recent workshop on leucocyte grouping in Leiden it appeared that the 8a antigen is, if not identical, closely linked to Mac, PlGrLy^{B1}, LA$_2$ and group 2 of Terasaki (J. J. van Rood

personal communication) and thus active not only to granulocytes (6) Further experience is required to determine if neonatal granulocytopenia is related to antibodies against only certain leucocyte antigens

3 Another possibility could be that the elimination of the child's leucocytes from the circulation which might be caused by the antibodies is compensated by an already existing extra vascular reserve (3, 20) or by an increased new formation Shulman (25) has shown that, after an injection of antibodies against leucocyte thrombocyte antigens, both cell types initially may decrease, then the granulocytes are rapidly restored whereas the number of thrombocytes increases more slowly The leucocyte antibody effect may be potentiated in certain cases assuming that the leucocyte production reserve of the patient is unusually low In our case the somewhat low granulocyte values even over a protracted period indicate that such a mechanism might have been active

4 A fourth possibility is that leucopenia and maternal antibodies are unrelated phenomena This possibility cannot be excluded in the present case Unfortunately the first sample for leucocyte antibody determination was drawn four weeks after birth Thus, it could not be proven that the maternal antibodies were produced before delivery

Summary

A case of granulocytopenia occurring in a newborn infant is described The mother s serum contained leucocyte antibodies active against the child s and the father's leucocytes The antibodies belonged to serological type anti 8a and consisted of IgG globulin The relation between the granulocytopenia and the serological findings is discussed

Acknowledgement

The authors want to thank Dr J J van Rood for his kindness to do the typing of the leucocyte antibodies and for his valuable suggestions concerning the manuscript

References

1 Braun E H H E Buckwold H E Emson and A V Russel Familial neonatal neutropenia with maternal leucocyte antibodies Blood 16 1745 1960
2 Buckwold A E H E Emson Acute neonatal neutropenia in siblings Canad med Ass J 80 116 1959
3 Craddock C G S Perry and J S Lawrence The dynamics of leukopenia and leukocytosis Ann intern Med 52 281 1960
4 Dausset J The leucoagglutinins Transfusion 2 209 1962
5 Dausset J and G Malinvaud Les agranulocytoses et granulopénies neonatales par isoimmunisation foeto maternelle Nouv Rev franc Hemat 4 154 1964
6 Dausset J and A Tangün Leucocyte and platelet groups and their practical significance Vox Sang 10 641 1965
7 Halvorsen K Neonatal leukopenia due to foeto maternal leucocyte incompatibility Acta Paediatrica Scandinavica 54 86 1965
8 Hitzig W H and R Gitzelman Transplacental transfer of leucocyte agglutinins Vox Sang 4 455 1959
9 Högman C F J Killander and S G O Johansson Identification of immunoglobulins with antibody activity fractionated by gel filtration Vox Sang 9 10 1964

10 *Jensen, K G* Transplacental passage of leucocyte agglutination occurring on account of pregnancy Danish med Bull 7 55 1960

11 Jensen K G Leucocyte antibodies in serums of pregnant women Vox Sang 7 454 1962

12 Jensen K G Leucocyte antibodies in serums of pregnant women Serology and clinic II Vox Sang 9 315 1964

13 Killander J and C F Hogman Fractionation of human blood group antibodies by gel filtration Scand J Clin & Lab Invest. 15 suppl 69 1963

14 Lalezari P., G E. Bernard Distribution of leukocyte antigens in various blood cells and body tissues Histocompatibility testing p 167 (Munksgaard Copenhagen 1965)

15 Lalezari P M Nussbaum, S Gelman T H Spoet Neonatal neutropenia due to maternal isoimmunisation Blood 15 236 1960

16 Lehndorff H Transitorische Granulocytopenie beim Neugeborenen Helv paediat Acta 6 1 3 1951

17 Luhby A L and L B Slobody Transient neonatal agranulocytosis in two siblings Transplacental isoimmunization to a leucocyte factor? A M A J of Diseases of Children 92 496 1956

18 Payne A R. The development and persistence of leucoagglutinins in parous women Blood 19 411 1962

19 Payne R. and M R Rolfs Foetomaternal leucocyte incompatibility J clin Invest 37 1756 1958

20 Payne A R., M R Rolfs M Tripp and J Weigle Neonatal neutropenia and leucoagglutinins Pediatrics 33 194 1964

21 Rood J J van Leucocyte grouping Thesis *University of Leiden* 1962

22 Rood J J van A Leeuwen van and J G Eernisse Leucocyte antibodies in pregnant women Vox Sang 4 427 1959

23 Rood J J van A Leeuwen van A M J Schippers W H Frederiks H Balner and J G Eernisse Leucocyte groups The normal lymphocyte transfer test and homograft sensitivity Histocompatibility testing p 37 (Munksgaard Copenhagen, 1965)

24 *Rossi J P and I K* Brandt *Transient* granulocytopenia of the newborn associated with sepsis due to shigella alkalescens and maternal leukocyte agglutinins J Pediat 56 639 1960

25 Shulman N R V J Marder L M Aledort, M C. Hiller Complementfixing isoantibodies against antigens common to platelets and leukocytes Transactions Association Am Physicians 75 89 1962

26 Slobody L. B H Abramson L S Loizeaux Agranulocytosis of the newborn infant J A M A 142 25 1950

27 Stefanini M H Mele D Skinner Transitory congenital neutropenia a new syndrome Amer J Med 25 749 1958

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5 Dausset J and G Malinvaud Les agranulocytoses et granulopenies néonatales par isoimmunisation foeto maternelle Nouv Rev franc Hemat 4 154 1964

6 Dausset J and Y Tangun Leucocyte and platelet groups and their practical significance Vox Sang 10 641 1965

7 Halvorsen K Neonatal leukopenia due to foeto maternal leucocyte incompatibility Acta Paediatrica Scandinavica 54 86 1965

8 Hitzig W H and R Gitzelman Transplacental transfer of leucocyte agglutinins Vox Sang 4 455 1959

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Table 1 Cancer death rates 1960—1962 (Age adjusted)

Male			Female		
Order	Site	Rate[1]	Order	Site	Rate[1]
1	Lung	33 4	1	Breast	22 2
2	Colon rectum	18 8	2	Colon rectum	17 0
3	Prostate	13 2	3	Uterus	13 2
4	Stomach	11 8	4	Ovary	7 6
5	*Pancreas*	8.2	5	*Stomach*	5 9
6	Leukemia	7 5	6	Leukemia	4.8
7	*Esophagus*	3 8	7	*Lung*	5 0
8	Kidney	3 2	8	Pancreas	4 8

[1] Rate per 100 000 population
Source American Cancer Society

dures potentially capable of prevent ing the development of leukemia in man have not yet been brought for ward although there are indications that certain types of leukemia in mice may be *partially protected against af* ter appropriate vaccination as was shown by Friend (8) in 1959

In the United States in children un der 15 years of age leukemia accounts for about 35 per cent of all neoplastic disease In the population as a whole (see table I) leukemia ranks in about sixth place among the various forms of neoplastic disease and the age ad justed mortality rate for leukemia is *about 6 per 100 000 population per* year This should be compared with an age adjusted mortality rate of approximately 125 per 100 000 popu lation per year for all types of cancer in the United States Thus leukemia accounts for about 5 per cent of all deaths from cancer in the United States or about 15 000 deaths per year

The problem is both large and unsolv ed and much more knowledge is needed than has so far been obtained

On several grounds it seems appro priate to suggest that the greatest de fect in current knowledge of leukemia in man has *to do* with causal factors particularly the primary incitants The importance of understanding the nature and the relative significance of causal factors needs no emphasis among those who are familiar with the remarkable record of the develop ment of effective control measures for other major *diseases which has been* accomplished during the last 40 years The control of most bacterial diseases with antimicrobial agents vaccines or toxoids of many viral diseases with viral vaccines and of several defi ciency diseases with vitamins or hor mones provides abundant evidence of *the crucial role that* knowledge of causal factors may play in the deve-lopment of effective controls

In the light of what has been learn ed since 1951 about the primary inci tants of leukemia in numerous species of animals especially mice chickens rats and hamsters as well as cats and possibly cattle it is not *surprising* that many investigators tend to lean to ward the hypothesis that leukemia in man may also be associated with cer tain viruses as yet unidentified This theory has received some indirect sup port as a result of the finding by Lie berman and Kaplan (12) in 1959 that leukemia induced by irradiation of mice could in some instances be transmitted by cell free filtrates and the more recent finding that *certain*

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Leukemia in man and Mouse

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To appreciate the significance and importance of leukemia in man, it is necessary only to recall that it continues to occur throughout the world's population and still causes severe illness and large loss of life, particularly during early years. In man its cause or causes are yet to be decisively demonstrated despite the fact that some 57 years have passed since Vilhelm Ellermann and Oluf Bang showed in 1908 that a form of leukemia of chickens could be transmitted by cell free filtrates and that some 14 years have gone by since Ludwik Gross demonstrated in 1951 that leukemia of mice was induced by a virus.

Its diagnosis in man is rarely accomplished until the disease is well advanced and has resulted in definite and often widespread pathological alterations which require the specific microscopic skills of the pathologist or hematologist for decisive recognition. Except for microscopy there are still no reliable or unequivocal laboratory procedures that serve as useful aids to diagnosis in patients. In contrast, it is now feasible to identify and even to classify directly by *in vitro* immunological procedures leukemic cells of several types from various species of animals, particularly mice.

The treatment of patients even by the most modern procedures, as initiated in 1948, employing combined or sequential chemotherapeutic agents including steroids, or radiation over long periods of time, has been of value in achieving remissions and extending the median time of survival from about four months to approximately 13 months as has been determined at the Memorial Hospital but only rarely leads to long term remissions lasting as long as five years.

At the International Congress of Hematology in 1964 Burchenal (4) reported that a total of 93 patients with acute leukemia were then known to have survived five years or more after diagnosis. More recently he has stated that the number of long term survivors has increased to about 104. The factors responsible for such long survivals are not yet known and apparently are not correlated with one or another treatment program. Proce

More recently the presence of similar particles has been reported in myeloid leukemia and certain other types of lymphoma. During the past year several reports (15, 16, 2) recorded the finding of such particles in centrifugally concentrated sediments from leukemic plasma. It has been held that these virus-like particles are approximately similar in size and form to the mouse and chicken leukemia viruses, but it is important to emphasize that no evidence for any biological activity has been presented and that several other workers have not obtained confirmatory results with comparable specimens.

In view of the uncertainties regarding the nature of such particles, the differences in their sizes and forms, and most importantly the fact that no biological activity has been reported, it seems premature to suggest that they represent viruses. Should they be found to satisfy all of the criteria required for the identification of viruses, it would still be necessary to determine what relation, if any, they may have to leukemia in man.

It should be emphasized that the finding of viruses in specimens from patients, especially those with neoplastic disease, does not of itself provide evidence for a relationship between the agent and the disease. Commensal viruses, or those that are simply "passengers" rather than "drivers," are both common and well known, particularly in the respiratory tract (80 known viral types) and intestinal tract (60 known viral types) of human beings. In patients with severe neoplastic disease such as leukemia it would not be surprising if such agents move about more readily than in normal persons and come to lodge in or even to infect neoplastic tissue.

During 1964, several workers in the United Kingdom and the United States reported the isolation of virus-like agents with definite biological activity from patients with leukemia (13) or the malignant lymphoma (6) of Africa, which has been suggested to represent a local counterpart of leukemia (5). In most instances these agents were recovered by the use of human cells in culture. There is considerable uncertainty about the nature of some of these agents, and they are still under active investigation. Some have been found to be either mycoplasma, i.e., pleuropneumonia-like organisms, or associated with the presence of mycoplasma organisms, which all too frequently are encountered in mammalian cell cultures that are maintained in series. One agent appears to have been identified as a reovirus (1).

Although it seems probable that certain of these agents were present in the specimens from which they were recovered, none has yet been shown to be oncogenic in animals or clearly to transform cells in culture. In contrast, the recognition of each agent has depended on its potentiality to produce cytopathic effects in cultured cells. Whether they should be considered as "passengers" or associated with leukemia or malignant lymphoma still remains uncertain. In a few instances (13, 6) tests for the presence of anti-

leukemogenic chemicals such as dimethylbenzanthracene appear also to activate latent or dormant leukemia viruses present in apparently normal host animals (17)

Among the three categories of primary incitants that have been found to be capable of inducing neoplastic disease in animals, i e, ionizing radiation, oncogenic viruses, and cancerigenic chemical compounds, extensive studies relating to two as possibly bearing on leukemia in man have been carried out

As a result of the continuing investigations of the Atomic Bomb Casualty Commission in Japan, there appears to be good evidence that a single very intense exposure to ionizing radiation ultimately led to an increased incidence of leukemia among the survivors and that there was a direct relationship between the large quantities of radiation received and the frequency of leukemia induction (3) It needs to be emphasized however that such very large amounts of radiation are not encountered under ordinary circumstances and that extrapolation of the data to background levels of radiation exposure may not be justified Nonetheless there remains an important question regarding the contribution, if any of continuing exposure to so called background radiation to the incidence of leukemia in man The fact that acute leukemia is more common among children under 15 years of age than among adults in the same environment is enough to cast considerable doubt on the theory that natural exposure to ionizing radiation plays a significant role as a primary incitant In addition, Dr Leonard Hamilton (11) has kindly provided computations based on currently acceptable hypotheses which suggest that background radiation during the normal life span would not be expected to account for more than about 3 per cent of the natural incidence of leukemia in man

Attempts to demonstrate viruses associated with leukemia in man have been reported frequently during the past 30 years and numerous intensive studies were stimulated by Gross discovery of the first mouse leukemia virus in 1951 (9) Despite several recent suggestive reports decisive evidence for an association between leukemia in man and viruses is still lacking Innumerable techniques and many different species of animals have been employed in such studies so far without yielding unequivocal results The use of newborn or thymectomized animals with deficient immune mechanisms as well as the employment of modern cell culture procedures, which have included various human cell types have not been rewarding and leukemia viruses of man if in fact they exist remain to be found

Several reports of the presence of virus like particles in the tissues or blood of patients with leukemia have appeared recently Beginning in about 1959 virus like particles have occasionally been found by Dmochowski and others with the electron microscope in the lymph nodes of patients with acute lymphatic leukemia (7)

Table II Forms of leukemia and lymphomas induced in mice and rats with mouse leukemia virus type A

LEUKEMIA	LYMPHOMAS
Lymphatic Leukemia	Lymphosarcomas
a) aleukemic	a) local lesion
b) leukemic	b) generalized
Stem cell Leukemia	Reticulum cell sarcoma
Myelogenous Leukemia	Hodgkin s like lesions
a) undifferentiated	
b) well differentiated	
Chloro Leukemia	
Erythroblastic Leukemia (atypical)	
Monocytic like Leukemia	

Gross, L Acta Haemat (Basel) 32 44 1964

in mice as well as a number in hamsters rats guinea pigs and rabbits

So far as is presently known the mouse leukemia viruses like those of the chicken leukosis sarcoma complex are of the RNA type range in diameter from about 70 to 110 mμ but are of variable size and shape and tend to be ether sensitive Infective RNA preparations have been obtained from tissues infected with the Moloney and Graffi viruses but similar procedures have failed to yield infective RNA from tissues infected with the Gross and Friend viruses

These viruses are thought to multiply in the cytoplasm but do not become fully mature until they reach the cell membrane which appears to take part in the completion of the particle The mature virus particle leaves the cell by a process called budding which does not usually lead to lysis or degeneration of the cell

The majority of mouse leukemia viruses are also pathogenic for rats and the Moloney virus is pathogenic for hamsters Newborn animals are the most susceptible presumably because of the immaturity of their immune responses and are preferred in studies on the recovery of leukemia viruses particularly with spontaneous chemically or X radiation induced leukemia Some of these agents are pathogenic for young animals and certain of them e g the Friend virus is also pathogenic for adult animals

Depending on their genetic make up mouse strains may vary widely in their susceptibility to leukemia viruses Other host factors such as hormonal balance and nutritional states may also affect susceptibility The chromosome number and karyotype of virus induced mouse leukemia cells appear not to differ markedly from those of control cells

In contrast to oncogenic viruses of the DNA type such as polyoma and Simian virus 40 the RNA containing leukemia viruses multiply readily in neoplastic tissues and considerable numbers of mature infective particles are produced This has facilitated their demonstration by both biological and physical procedures. Despite the concentrations that can be obtained in purified preparations the specific antigenicity of leukemia viruses as such tends to be relatively weak and is particularly poor with Gross virus Specific antiviral sera can be obtained but they are not often of high titer and may give irregular results in cross

bodies in the serum of patients have yielded results suggesting that antibodies against the agents were present in some patients but not in control sera Such evidence, even if confirmed, would not itself establish an association between the agents and the disease, although it would indicate that an immune response had occurred, presumably as a result of an infective episode Unfortunately, at the present time, it is much easier to show that a given virus or other infective agent is not causally associated with leukemia in man than it is to demonstrate that it may serve as a primary incitant of the disease

In contrast to the inadequacy of current knowledge of the etiology of leukemia in man, much is known of the etiology of leukemia in the mouse Because the disease in the mouse may serve as a useful and stimulating model of the disease in man, the subject merits a brief review

Many workers now incline to the view that mouse leukemia regardless of morphological type or strain of mice is, in fact, a viral disease Relative to naturally occurring neoplastic disease in animals mouse leukemia and the chicken leukosis sarcoma complex appear to be more fully understood than any others Some 14 leukemogenic virus strains have been recovered from mice since the first mouse leukemia virus was discovered by Gross in 1951 It is doubtful that these are all definitely different viruses and it seems more probable that several may represent variants of one stem virus On immunological analysis, for example, it appears that three of the viruses, i e, the Friend Moloney, and Rauscher agents, form a cross reacting group and are closely related to one another (14) They are however, not related immunologically to Gross virus which, it appears, should now be regarded as the mouse virus that typifies naturally occurring mouse leukemogenic viruses

The Gross virus leads to the appearance of a cell antigen which is distinct from other known antigens found in mouse leukemia This antigen appears to characterize all leukemias, regardless of morphological type, induced by the Gross virus as well as many forms of spontaneous mouse leukemia and those induced by chemical compounds or by X radiation In contrast, cell antigens of the Friend Moloney, Rauscher type have not been found in spontaneous chemically or X-radiation induced leukemia They appear only in leukemias induced by inoculation of these viruses (14)

On morphological grounds many different forms of leukemia or lymphoma may be induced in mice or rats by the Gross virus Gross (10) recently compiled a list (see table II) of some 12 forms of neoplastic disease that have been induced by the Gross mouse leukemia virus which he now designates as Type A The variety of morphological types of neoplastic disease which may result from infection with a single virus is not unique and, in fact has been exceeded by the even more striking potentialities of polyoma virus which has induced more than 20 different types of solid tumors

References

1 Bell T M Massie A Poss M G R. & Williams M C. Isolation of a reovirus from a case of Burkitt s lymphoma Brit Med J 1 1212—1213 1964

2 Benyesh Melnick M Smith K O & Fernbach D J Studies on human leukemia III Electron microscope findings in children with acute leukemia and in children with infectious mononucleosis J Nat Cancer Inst 33 571—579 1964

3 Brill A B Tomonaga M & Heyssel R M Leukemia in man following exposure to ionizing radiation A summary of the findings in Hiroshima and Nagasaki and a comparison with other human experience Ann. Intern Med 56 590—609 1962

4 Burchenal J H Long term remissions of acute leukemia spontaneous and induced Scand J Hemat 1 4—56 1965

5 Dalldorf G Lymphomas of African children with different forms or environmental influences J A M A 181 1026—1028 1962

6 Dalldorf G & Bergamini F Unidentified filtrable agents isolated from African children with malignant lymphomas Proc Nat Acad Sci 51 263—265 1964
Dmochowski L Viruses and tumors Science 133 551—561 1961

8 Friend C Immunological relationships of a filterable agent causing leukemia in adult mice J Exp Med 109 21—28 1959

9 Gross L "Spontaneous" leukemia developing in C3H mice following inoculation, in infancy with AK leukemic extracts or AK embryos Proc Soc. Exp Biol Med 76 27—32 1951

10 Gross L. How many different viruses causing leukemia in mice? Acta Haemat. (Basel) 32 44—62 1964

11 Hamilton L D Personal communication, 1964

12 Lieberman, M & Kaplan, H S Leukemogenic activity of filtrates from radiation induced lymphoid tumors of mice Science 130 387—388, 1959

13 Negroni G Isolation of viruses from leukaemic patients Brit Med J 1 927—929 1964

14 Old, L J & Boyse E A Antigens of tumors and leukemias induced by viruses Fed Proc 24 1009—1017 1965

15 Porter G H III Dalton A J Moloney J B & Mitchell E Z Association of electron dense particles with human acute leukemia J Nat Cancer Inst 33 547—552 1964

16 Smith K O Benyesh Melnick, M & Fernbach D J Studies on human leukemia II Structure and quantitation of myxovirus like particles associated with human leukemia J Nat Cancer Inst. 33 557—563 1964

17 Toth B Development of malignant lymphomas by cell free filtrates prepared from a chemically induced mouse lymphoma Proc Soc Exp Biol Med 112 873—875 1963

neutralization tests This has made immunological studies on the agents themselves difficult to evaluate

Inactivated leukemia virus vaccines have been prepared and, with certain agents, e g , the Friend virus, have been shown to be moderately effective in producing immunity against leukemia induced by the same virus

Despite the weak antigenicity of leukemia viruses, the leukemic cells of animals infected with such viruses contain new antigens which make it feasible to distinguish certain immunological types of mouse leukemia by *in vitro* procedures The cytotoxic test which has been intensively studied by Old and Boyse (14), has the advantages of simplicity and speed when appropriate immune sera are available Immune sera may be produced either by growth and rejection of transplanted isogenic leukemia or infection with a leukemogenic virus in adult mice In the presence of guinea pig complement such sera are cytotoxic for leukemia cells which possess new antigens homologous with those of the leukemia used for immunization or the leukemia induced by the virus used for immunization Because of the use of isogenic leukemic tissue there is no immune response to the transplanted cell antigens other than those new antigens which characterize the leukemic cells

Old and Boyse (14) have found five distinct cell antigens demonstrable by the cytotoxic test in mouse leukemias Three of the antigens are associated with known leukemogenic viruses i e 1) Gross virus, 2) Friend, Moloney, and Rauscher viruses, and 3) mammary tumor agent Two other antigens are now known to be associated with the presence of leukemogenic viruses and may take their origin from the genetic material of the leukemia cell Soluble circulating antigens, separable from infective virus and with the same specificity as the cell antigens, have been demonstrated in the plasma of mice with leukemia induced by the Gross, Friend Moloney, or Rauscher viruses

It seems possible and certainly is to be hoped that these solid advances in knowledge of the immunology of leukemia in the mouse may have applications to leukemia in man Although there are serious genetic barriers which greatly impede comparable studies in human beings, it appears that they may not be insurmountable and that a search for analogous cell antigens in leukemia in man may be feasible

As a concluding comment it seems appropriate to emphasize that there are many close similarities between leukemia of the mouse and leukemia of man In the light of what has been learned of the etiology of mouse leukemia, it would be difficult to defend the view that viruses are improbable candidates for a role as primary incitants of human leukemia It is to be expected that further studies will ultimately provide decisive answers which will either support or disprove the hypothesis that viruses are associated with leukemia in man

phytohaemagglutinin (PHA) and for 72 hours with PHA

1 *Two male patients with similar alteration in karyotype (J H and T Y)*

Clinically the two patients were unlike Neither had received X ray therapy both had been treated with busulphan. T Y had been treated since 1951 *his clinical condition* was well controlled initially with arsenic and urethane later by intermittent courses of busulphan *In February 1965 when the chromosomes* were examined the patient felt well but the liver and the spleen were enlarged *and blast cells had appeared in the blood* The terminal illness was rapid and the patient died aged 61 on March 5th 1965 after *14 years in the chronic stage*

J H in contrast had a longer terminal illness following satisfactory control for 2 years by *continuous busulphan therapy* Blast cells appeared in the peripheral blood and the haemoglobin concentration began to fall. Mercaptopurine was ineffective Four months later (January 1965) he developed jaundice fever and a perineal abscess He was deeply pigmented and had lost weight The haemoglobin level continued to fall in spite of blood transfusions He died on March 21st 1965 aged 57

In Romanowsky stained bone marrow films *in both cases the majority of the cells were* blast cells

The karyotypes were studied in cells grown in blood cultures In T Y 13 of the 14 cells examined contained the Ph^1 chromosome only one of the Ph^1 positive cells was *normal in respect of* the remaining chromosomes Twelve cells contained two extra chromosomes in the C group and 10 of them lacked *one* chromosome in the E group

In J H 13 of the 15 cells examined contained the Ph^1 chromosome All 13 also contained extra C group chromosomes one in two cells two in nine cells and four in one cell Eleven lacked an E group chromosome The most deranged *cell had four extra C group* chromosomes one extra G and lacked two E group chromosomes Thus the chromosome complement varied in the cells in both cases from 45 to 49

The similarity in chromosome *rearrangement in* these two cases was not associated with any similarity in the clinical evolution of the terminal blast cell changes

Pedersen reported two cases of this *type of change in karyotype in* young men (6) In one case the abnormal cell line appeared 2 1/2 months before death and in the other 3 1/2 years after the diagnosis of CGL and 6 *months before death* The karyotype changes in these four cases cannot be assumed to be identical The morphological identification of individual members of the same group of chromosomes is not possible and numbers 17 and 18 of the E group cannot be identified with certainty

2 *Three males with an XO chromosomal complement in the bone marrow cells*

Two of these patients, E I (case no 22) (7) and *D J* did not show any additional karyotype changes in the terminal stages

E I who had four sons was 58 when CGL was diagnosed in 1957 After an initial course of radiotherapy to the spleen the disease remained well controlled by continuous busulphan *therapy* Mercaptopurine was substituted towards the end of 1964 because of *anorexia* weight loss, deep pigmentation and fall in the blood *pressure and the haemoglobin* concentration In January 1965 *blast cells*

Department of Clinical Research The Royal Marsden Hospital, and
the Chester Beatty Research Institute Institute of Cancer Research[1]

Chromosome Changes in the Terminal Stages of Chronic Granulocytic Leukaemia

By S D LAWLER and D A G GALTON

The bone marrow cells of classical chronic granulocytic leukaemia (CGL) almost always contain a characteristic abnormal small acrocentric ("Philadelphia", Ph^1) chromosome (no 21, Denver nomenclature, 5, 2) Some patients who have Ph^1-positive cells are not, however, clinically or haematologically typical though the Ph^1-negative cases are far more heterogeneous (4)

The Ph^1 chromosome is confined to the bone marrow cells, the cells in skin and lymphocyte cultures are normal In the polyploid cells (probably megakaryocytes) of bone marrow the number of Ph^1 chromosomes corresponds to the ploidy The evidence relating to erythrocyte precursors is indirect In a typical case more than 90 % of the bone marrow cells are Ph^1 positive whatever the ratio between erythropoietic and granulopoietic cells

In the chronic phase of the disease apart from the presence of the Ph^1 chromosome, the karyotype is usually normal The bone marrow cells of a few affected males not necessarily clinically similar, are XO Ph^1 positive XY Ph^1 positive cells may also be present (1, 3, 7) The cells in lymphocyte and fibroblast cultures, however, have been found to be normal XY and some of these patients have had male children CGL has also been described in a sex chromosome mosaic of the XY/XXY type (3)

Sometimes, additional chromosomal anomalies appear in the terminal stages of the disease These changes tend to be unique for each patient We now examine the clinical circumstances in which these changes have arisen

Some of our 8 patients (all males) studied during the chronic phase were previously described (7) The absence of females is almost certainly fortuitous Unless otherwise stated the chromosomes in bone marrow were examined by a modified "direct" technique (8) Blood cultures were usually made for 24 48 and 72 hours without

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chromosome was not the first additional abnormality, because only one was present in the earliest stage of the blast cell crisis. The original population was eventually replaced by even more abnormal cells. The treatment with dibromomannitol destroyed many blast cells but after treatment cells appeared even more deranged than those present before. The patient died on July 30th 1965.

Thus the clinical course during the chronic phase, and the events of the terminal illness of these three male patients with an initially similar Ph^1 positive XO karyotype were very different. In two cases the chronic phase was benign and well controlled for several years but in one of them the terminal phase was acute and associated with the appearance of several abnormal karyotypes showing evidence of variation and selection in the population of blast cells, while in the other the terminal illness was protracted and not associated with any change in karyotype. The third patient had a short poorly controlled chronic phase, a long terminal illness with blast cell increase in the blood and bone marrow but no change in the original karyotype.

3 *Extremely rapid terminal changes accompanied by karyotype changes not involving the entire cell population*

H.O., a Jamaican, was 59 when CGL was diagnosed in 1963. There was gross splenic enlargement and generalised but minimal lymphadenopathy; the haematological picture was that of classical CGL. The karyotype studied in a blood culture was Ph^1 positive and showed no other abnormalities. He responded well to busulphan but it was difficult to stabilise the leucocyte count.

From mid December the haemoglobin concentration, hitherto well maintained, fell rapidly and on January 12th 1965 50 per cent of the bone marrow cells were blasts; only occasional blast cells were seen in blood films. The karyotypes of 13 bone marrow cells (January 12th 1965) all contained the Ph^1 chromosome. Six had no other abnormalities whilst seven lacked two C group chromosomes and had one extra D group chromosome; one of these seven cells also had an extra C-group chromosome and another two extra E group ones.

It is probable that the cells with the abnormal karyotypes were the blast cells seen in the Romanowsky stained films, the cells with the original karyotypes being the residual cells of the chronic phase. The terminal changes were extremely rapid; the patient died on February 16th 1965.

4 *Two patients who showed the development of successful clones of abnormal cells in the terminal stages*

J.D. was 40 when CGL was diagnosed in April 1963. He responded well to busulphan until June 1964. He never received radiotherapy. A bone marrow preparation (July 1964) showed only Ph^1 positive otherwise normal cells. The patient then had a large liver and spleen, and pericardial and pleural effusions. His condition deteriorated and he died on October 4th 1965.

In a blood culture (October 3rd 1964) the most frequent chromosome

appeared in the blood A chest infection in March 1965 responded to antibiotics but he became weaker and developed signs of a spinal lesion A bone marrow preparation in January 1965 produced few dividing cells all were XO Ph¹ positive Romanowsky stained films showed granulocytic hyperplasia without an excess of blast cells

D J was 43 when CGL was diagnosed in 1963 He had previously had malaria with enlargement of the spleen which however was not felt in 1963 The leucocyte count was 160 000 per cu mm of which 320 were blasts He responded to splenic X irradiation but relapsed rapidly Little improvement was subsequently obtained with busulphan and prednisone therapy He had recurrent attacks of gout He remained anaemic and died in December 1964 18 months after the diagnosis had been made satisfactory clinical and haematological control not having been achieved

Blast cells up to 14 500 per cu mm were present in the blood during the last 16 months of life In Romanowsky stained films large and small blasts with distinctive morphological characteristics were found Only the large blasts fell in number during busulphan therapy This dual population of cells was not reflected in karyotype differences The dividing cells were few in number in the various preparations made but only XO Ph¹ positive cells were ever found in blood cultures or bone marrow

D C (no 23) (7) was 27 when CGL was diagnosed in 1957 The disease showed the classical clinical and haematological features of CGL and was well controlled for 7½ years by continuous busulphan therapy He received no radiation therapy but mannomustine was given for a month in 1957 During 1962 the bone marrow cells analysed all contained the Ph¹ chromosome and lacked the Y chromosome The blood lymphocytes and the skin fibroblasts all had a normal male karyotype and were Ph¹ negative Serial examinations of the chromosomes were made during the terminal stages The changes were manifold but all the cells lacked a G group chromosome (presumably Y) and contained at least one Ph¹ chromosome The additional changes were progressive and appeared to be superimposed on the cell line of the chronic phase

In February 1965 the haemoglobin concentration began to fall On May 20th 1965 the marrow contained 84 per cent of blast cells and rearrangements were already present in the chromosomes in both blood and bone marrow The karyotype of 4 of the 21 cells analysed was the same as that found in the chronic phase (XO Ph¹ positive) The remaining 17 cells showed additions of the D or F group and loss of E group chromosomes The chromosome number varied from 44 to 49

On May 24th 1965 busulphan was replaced by dibromomannitol, administered until June 10th 1965 The blast cell count fell rapidly and in a blood culture (June 10th) only severely damaged chromosomes were found

On June 24th 1965 every XO Ph¹ positive cell in a blood culture now showed additional changes, five of the 10 cells analysed had two Ph¹ chromosomes Of the four cells with 48 chromosomes, two had the same chromosome complement including two Ph¹ chromosomes and the two with 49 chromosomes resembled them but in addition had an extra C group chromosome The remaining four cells had 45 chromosomes In a fourth culture (July 8th 1965) all the cells with more than 46 chromosomes now had two Ph¹ chromosomes four cells had 48 chromosomes that were identical with one another but differed from the cells in the previous culture having 48 chromosomes in having an extra C group chromosome instead of an F Thus a series of rearrangements were present basically similar but varying amongst themselves Possibly a cell line with 48 chromosomes was beginning to dominate The appearance of a second Ph¹

chromosome was not the first additional abnormality, because only one was present in the earliest stage of the blast cell crisis The original population was eventually replaced by even more abnormal cells The treatment with dibromomannitol destroyed many blast cells but after treatment cells appeared even more deranged than those present before The patient died on July 30th 1965

Thus the clinical course during the chronic phase and the events of the terminal illness of these three male patients with an initially similar Ph¹ positive XO karyotype were very different In two cases the chronic phase was benign and well controlled for several years but in one of them the terminal phase was acute and associated with the appearance of several abnormal karyotypes showing evidence of variation and selection in the population of blast cells while in the other the terminal illness was protracted and not associated with any change in karyotype The third patient had a short poorly controlled chronic phase a long terminal illness with blast cell increase in the blood and bone marrow but no change in the original karyotype

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Table I A J Blood Counts and Chromosome Studies

Date	Total WBC/ cu mm	% Blasts in PB	Preparation	Chromosome Number							No of cells
				46	47	48	49	50	51	52	
29 7 63	77 000	15 %	PB 24 hrs	1	0	1	10	12	1	0	25
			PB 26—28 hrs	2	2	1	4	22	1	0	32
13 8 63	8 000	5 %	PB 40 hrs	4	6	2	10	5	1	0	28
15 8 63	7 600	5 %	BM Dir	0	0	2	2	4	0	1	9
			BM 24 hrs	2	0	2	3	16	1	0	24
19 9 63	5 200	7 %	PB 24 hrs	1	0	1	3	12	1	0	18

WBC leucocyte count PB peripheral blood BM bone marrow

number was 48 (23 out of 50 cells) Ten of the 23 cells with 48 chromosomes that were analysed or karyotyped had two Ph^1 chromosomes and an extra chromosome in the C group A few cells typical of the chronic phase remained Most of the cells with more than 46 chromosomes had two Ph^1 chromosomes This acquisition of a second Ph^1 chromosome in the terminal stages was described by Court Brown (3) In addition there is evidence here for the emergence of a stable clone of cells

A J a West Indian was 27 in 1962 when the diagnosis of CGL was made The spleen was irradiated following a transfusion and a short course of busulphan The response was poor and busulphan therapy was resumed The level of haemoglobin well sustained until May 1963 then fell the spleen enlarged rapidly and the blast cell count in the blood rose steeply From July 25th 1963 he received blood transfusions intravenous injections of Mannitol Myleran and splenic X irradiation with little benefit and died on October 2nd 1963 The disease was never controlled by radiotherapy or busulphan and the terminal blast cell phase began less than one year from the time of diagnosis At presentation the blood and bone marrow findings were those of classical CGL a blood culture (November 15 th 1962) showed Ph^1 positive normal male cells

Serial chromosome studies were made during the terminal illness Table I shows the chromosome counts obtained the leucocyte counts and the percentage of blast cells The cell lines with 49 and 50 chromosomes maintained a constant karyotype throughout the illness in contrast to the progressive and variable changes observed in D C The chromosome constitution of the majority of the cells in each culture with 49 chromosomes was A6 B4 C16 D6 E6 F6 G5 (1 Ph^1 positive chromosome) that of the cells with 50 chromosomes was identical except for the presence of a second Ph^1 chromosome (Fig 1) Varying the cultural conditions scarcely altered the proportion of cells with 49 and 50 chromosomes but the 40 hour blood culture contained a higher proportion of cells with 49 chromosomes than any other Nor did the proportion change throughout the illness and only 5 cells out of the 136 observed had more than 50 chromosomes One



Fig 1 Karyotype of cell with 50 chromosomes from patient A J

of the cells with 51 chromosomes had the same analysis as that of the line of cells with 50 chromosomes with a second extra C group chromosome

In this case as in that of D C serial chromosome studies showed that abnormalities in addition to the presence of the Ph[1] chromosome appeared in the terminal blast cell phase In D C the derangements were progressive and appeared to indicate instability in a cell population whereas the cells of A J showed the dominance of two stable and apparently related cell lines These lines persisted over a period of 7 weeks in spite of changes in the blast cell count brought about by the treatment administered

Comment

The terminal stage of CGL affords an opportunity of studying the accumulation of additional chromosomal abnormalities in a cell population in different patients already known to have a characteristic and constant abnormality All the secondary changes observed have in fact occurred in cells carrying the primary abnormality that is the Ph[1] chromosome The changes have consisted in additions and losses in the normal chromosome complement rather than structural abnormalities and there has been a tendency to hyperdiploidy

The earliest as well as the successive secondary changes varied both between patients and sometimes within the population of cells in an individual This does not preclude the possibility that the terminal changes are initiated by mutation of an individual cell not reflected in karyotype abnormalities Indeed in two cases the blast cell crisis was not associated with increasing aneuploidy but terminally only small numbers of dividing cells could be examined In both cases the Y chromosome was absent from the marrow cells throughout the disease

Similar additional abnormalities have recurred in different patients which perhaps suggests that they confer selective advantages to the cells carrying them The tendency for certain cell lines to persist to dominate or to replace others indicates that an abnormality with survival value must also be stable

The abnormalities observed could not be correlated with the duration of the chronic phase or with the rate of progression of the terminal phase

Acknowledgements

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The Blood Research Foundation Center[1] Hospital of Tortona Italy
Director G Astaldi

Is the Defective Reaction to Phytohaemagglutinin shown by the Lymphocytes from Lymphocytic Leukaemia Depending on their Innate Structure or on Plasmatic Characteristics?

By G ASTALDI M D G COSTA Ph D R AIRO M D
and N DUATE Res Tech

Alterations in the composition of blood proteins are a rather common occurence in disorders of the histo lymphatic system About two decades ago J Waldenstrom described a new syndrom caracterized by the presence of an elevated amount of macroglobulins in the *serum and of a "lymphoid"* metaplasia in the haemopoietic organs (1) This syndrom has since been named *Waldenström s Macroglobuline mia*

In recent years hypogammaglobulinaemia with impaired immuno response has been reported as a frequent feature of Chronic Lymphocytic Leukemia (CLL) (2) On the other hand it has been proved in different laboratories that peripheral blood lymphocytes from CLL patients give a low percentage of blasts when stimulated with Phytohaemagglutinin (PHA) in tissue culture (3—9) Among the lymphoid cells we obtained a percentage of blasts of 9±3 % in CLL after 72 hours of culture with PHA as compared with 68 5±5 % in healthy persons and of 69±6 % in chronic granulocytic leukaemia

The defective response shown by CLL lymphocytes can be due either to the intimate biology of these cells (*their age their leukaemic condition* their immuno incompetence and therefore their PHA unresponsiveness) or to some inhibitory factors present in their plasma or in the culture system In order to bring some light in this problem a series of experiments was undertaken by our group in this paper we discuss the results concerning the observation that CLL plasma is capable of inhibiting the blastic development of lymphocytes from healthy persons when these lymphocytes are submitted to the PHA *stimulation in* tissue culture

Material and methods

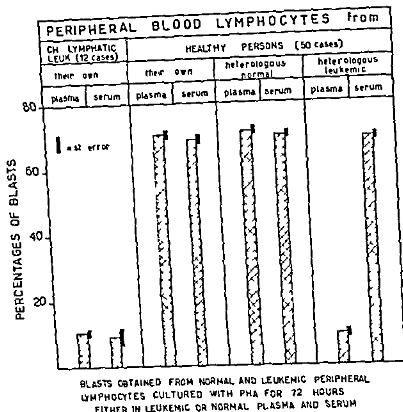
In these experiments the cultures were set up according to the technique

[1] Supported by The Blood Research Foundation Washington D C USA

tals who have referred patients to us and we also thank Mrs I L Hansteen and Mrs P Dodd for assistance with the chromosome studies

References

1 Atkin N B and Taylor M C (1962) Cytogenetics *1* 97
2 Baikie A G Court Brown W M Buckton K E Harnden D G Jacobs P A and Tough I M (1960) Nature (Lond) *188* 1165
3 Court Brown W M and Tough I M (1963) Adv Cancer Res *7* 351
4 Krauss S Sokal J E and Sandberg A A (1964) Ann int Med *61* 625
5 Nowell P C and Hungerford D A (1960) J nat Cancer Inst *25* 85
6 Pedersen B (1964) Acta path microbiol scand *61* 497
7 Speed D E and Lawler S D (1964) Lancet *1* 403
8 Tjio J H and Whang J (1962) Stain Tech *37* 17



BLASTS OBTAINED FROM NORMAL AND LEUKEMIC PERIPHERAL LYMPHOCYTES CULTURED WITH PHA FOR 72 HOURS EITHER IN LEUKEMIC OR NORMAL PLASMA AND SERUM

Fig 1

cytes were cultured with normal plasma In other words normal lymphocytes when cultured with CLL plasma clump like CLL lymphocytes

d) finally the lymphocytes from CLL patients cultivated in medium added to their own plasma and in medium added to their own serum developed a percentage of blasts of 10 0± 4 4 and 8 5±6 3 respectively

2 — The results of the experiment e) are summarized in the graphs of Fig 2 It appears that peripheral blood lymphocytes from CLL patients gave very low percentages of blasts when cultured with plasma from healthy persons and that the addition of plasma from healthy persons did not modify the usual PHA unresponsiveness of cultured CLL lymphocytes also when these lymphocytes were previously washed 1—4 times In fact the percentages of blasts obtained after 72 hours of tissue culture with PHA were on an average

a) 6 9±5 2 when the lymphocytes from CLL were cultured without previously washing in medium added to plasma from healthy people and 5 3± 3 3 when the same CLL lymphocytes were added to the CLL plasma

b) 7 4±5 7 7 9±7 2 9 4±8 3 8 6±

described elsewhere (10), and the percentage of blasts among the lymphatic cells was determined after 72 hours of tissue culture The investigation consisted of the following three experiments

a) *Lymphocytes from healthy people cultured in a medium containing PHA and plasma from CLL-patients* — Peripheral blood lymphocytes from 50 healthy persons were cultivated in NCTC 109 (Microb Ass) added to PHA (prepared by us in our Lab) (11) and divided in three lots The first lot was added to autologous plasma (20 %), the second lot to heterologous normal plasma (20 %), and the third lot to plasma from 6 different CLL patients (20 %) At the same time the peripheral blood lymphocytes from these CLL patients were cultivated in the same NCTC 109 with PHA added to 20 % of their own plasma

b) *Lymphocytes from healthy persons cultured in a medium containing PHA and serum from CLL-patients* — The details of this experiment were similar to those of experiment a) with the only difference that instead of plasma from CLL patients serum from the same 6 CLL patients was used

c) *Lymphocytes from CLL-patients washed and then cultured in a medium containing PHA and plasma from healthy people* — Peripheral blood lymphocytes from each of 6 CLL patients were divided in two lots the first lot was cultivated in NCTC 109 added to PHA and to heterologous normal plasma (20 %), the second lot was cultured in the same medium with PHA and added to autologous (leukaemic) plasma (20 %) this was used as control For each of these two lots, 5 series of culture flasks were prepared, in the first, second, third and fourth series the lymphocytes were cultivated after washing with pure culture medium 1, 2, 3 and 4 times, respectively, in the fifth series the lymphocytes were cultured without any previous washing

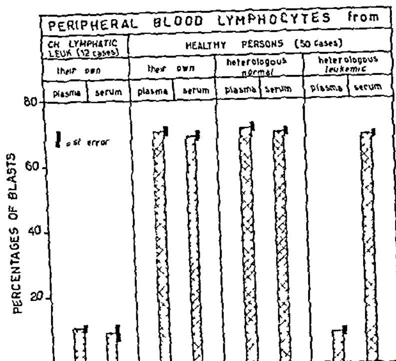
Results

1 — The results of the experiments a) and b) are recorded in the graphs of Fig 1 It is clearly evident that the addition of plasma from CLL patients to cultures of lymphocytes from healthy persons inhibits the blastic development of these cells, whereas the addition of the same amount of serum from the same patients has no effect In fact, blood lymphocytes from healthy people after 3 days of tissue culture with PHA developed the following percentages of blasts

a) 71 5±6 0 and 68 5±7 9, when added to their own plasma and to their own serum respectively

b) 73 5±3 5 and 70 5±3 0 when added to a *heterologous normal* plasma and to a heterologous normal serum respectively

c) only 10 0±2 7 when added to plasma from CLL patients *but 69 5±2 0* when added to serum from the same CLL patients Moreover these lymphocytes from healthy persons cultivated with CLL plasma appeared much more densily clumped together then when the same normal lympho



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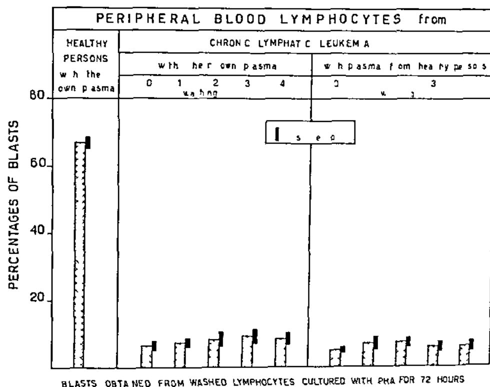


Fig 2

5.1 when the CLL lymphocytes were washed 1, 2, 3 and 4 times and then cultured with normal plasma and 7.0±5.9, 7.5±5.5, 5.7±5.9 and 6.6±4.8 when the same washed lymphocytes were cultured with their own plasma. In other words the number of blasts obtained in the experimental and in the control groups was of the same order of magnitude.

Discussion

The defective reaction of lymphocytes from CLL to PHA stimulus in tissue culture is a well documented phenomenon, whereas the causes why the lymphocytes of these patients give so low percentages of blasts in culture with PHA are not yet definitely established.

Bernard et al. (7) and Oppenheim et al. (12) admit that in CLL there exists an inverse relation between the total leucocyte count in the peripheral blood and the number of blasts obtained in culture with PHA. On the other hand Elves et al. (13) believe that only the lymphocytes from cases of CLL with hypogammaglobulinaemia give a low percentage of blasts in tissue culture with PHA, whereas the lymphocytes from CLL patients with normal amount of gammaglobulin should give an almost normal percentage of blasts. In our experiments we have not observed any relationship between the total leucocyte count and the percentage of blasts obtained in culture nor between the gammaglobulin content and the blastic development of the lymphocytes of our patients, as 9 of the 12 cases

of CLL reported showed around normal values of gammaglobulin and the percentages of blasts were low in all of them. Moreover it has been observed that lymphocytes from cord blood, as well as lymphocytes from hypogammaglobulinaemic and from agammaglobulinaemic patients give very high percentages of blasts in cultures with PHA (14). Thus we have been tempted to relate the defective reaction to PHA shown by CLL lymphocytes to the fact that these cells tend to clump in the culture flasks preventing in this way the surface action of PHA (10).

The defect of response to PHA shown by CLL peripheral blood lymphocytes in tissue culture could depend also on the possible existence of two clones of lymphocytes with different kinetics, different immunological competence, different sites or origin. In fact peripheral blood lymphocytes come from different sources: the majority of them from the mantle zones around the lymphoid germinal centers and from the medullary cords and pulp of lymph nodes and spleen, whereas the remaining blood lymphocytes come from germinal centers, thymus cortex and bone marrow. The former seems to be the immuno-competent cells and the latter are believed to be the immuno-non-competent ones (15, 16). It is possible to hypothesize that in CLL a clone of lymphocytes prevails which comes from those sites (one or more) in which the lymphocytes are immuno-non-competent and that the defective reaction to PHA shown by the lymphocytes in CLL is just an exaggeration of a situation present in healthy persons where also, as it is well known, some lymphocytes are PHA non responsive.

The results of our experiment a), reported above, show very clearly that the plasma from CLL patients added to the cultures of peripheral blood lymphocytes from healthy people inhibits the blastic development of these cells. This observation is in contrast with those made by Schreck (4), Robbins (14) and Oppenheim (12) who reported that the addition of plasma from CLL patients to the cultures of lymphocytes from healthy persons do not inhibit the blastic development of these cells. On the other hand differences in technique and in the amount of CLL plasma added to the culture medium existing between our experiment and those of the above mentioned authors make it difficult to compare the results.

The observation that lymphocytes from healthy persons tend to clump when cultivated in a medium containing plasma from CLL suggests that the defective response to PHA shown by normal lymphocytes in these conditions could be due to the fact that PHA cannot reach the cell surface and initiate the blastic development just as it happens for CLL lymphocytes. On the contrary the third part of our investigation shows that the deficient responsiveness of CLL lymphocytes to PHA in tissue culture could primarily be due to an intimate cellular defect rather than to the action of the inhibiting CLL plasma. In fact CLL lymphocytes previously washed several times and then cultured with PHA in

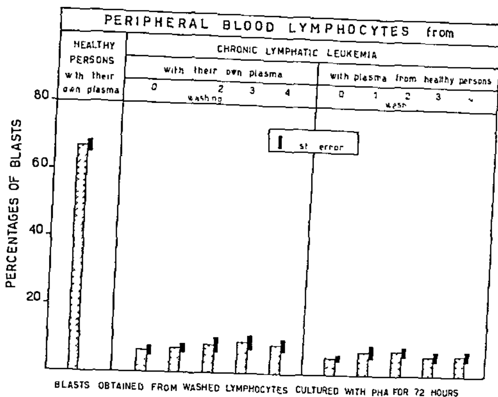


Fig 2

5 1, when the CLL lymphocytes were washed 1 2 3 and 4 times and then cultured with normal plasma and 7 0±5 9, 7 5±5 5, 5 7±5 9 and 6 6±4 8 when the same washed lymphocytes were cultured with their own plasma In other words the number of blasts obtained in the experimental and in the control groups was of the same order of magnitude

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Peripheral blood lymphocytes from CLL patients washed 1—4 times and then cultured in a medium added to plasma from healthy persons did not change their usual hyporesponsiveness to the PHA stimulation in tissue culture

Finally the CLL blood lymphocytes reacted normally to the PHA stimulation in culture after the spleen irradiation of the CLL patients

The above summarized results are discussed

References

1 Waldenstrom J Incipient myelomatosis or essential" hyperglobulinemia with fibrinogenopenia A new syndrome? Acta Med Scand 117 216 1944

2 Stefanovic S Stojkovic I Dimic B Ristic M et Banicevic B L immunité dans les lymphomes malins Presented at the 10th Congr of the Europ Soc Haematol *Strasbourg (France)* 1965

3 Nowell, P C Differentiation of human leukemic lymphocytes in tissue culture Exp Cell Res 19 267 1960

4 Schrek R and Rabinowitz, Y Effect of phytohemagglutinin on rat and normal and leukemic human blood cells Proc Soc exp Biol Med 113 191 1963

5 Quaglino D Cytochemistry in relation to short term tissue culture Proc 9th Congr Europ Soc Haemat (Lisbon 1963) — S Karger Basel/New York Part II/1 p 112 1963

6 *Quaglino D and Cowling D C Cytochemical studies on cells from chronic* lymphocytic leukaemia and lymphosarcoma cultured with Phytohaemagglutinin *Brit J Haemat* 10 358 1964

7 Bernard C Geraldes A et Boiron M *Action de la phytohémagglutinine in vitro* sur les lymphocytes de leucémies lymphoides chroniques Nouv Rev Franc Hemat 4 69 1964

8 Robbins J H Human peripheral blood in tissue culture and the action of phytohemagglutinine Experientia 20 164 1964

9 Astaldi G Das Phytohamagglutinin und das Problem des Lymphozyten Med Klinik 59 4 1964

10 Astaldi G Sauli S Airò R, Rallo L and Costa G With references to the effect of phytohemagglutinin on lymphocytes from different leukemias Texas Rep Biol Med 23 569 1965

11 Astaldi G Strosselli E. e Sauli S Le cellule emiche nella ricerca citogenetica Haematologica (Suppl) 47 1 1962

12 Oppenheim, J Whang J and Frei E Immunologic and cytogenetic studies of chronic lymphocytic leukemic cells Blood 26 121 1965

13 Fives W Stuart R and Israels M Failure of lymphocytes from hypogammaglobulinaemic subjects to transform in culture Brit Med J ii 1051 1964

14 Robbins J Tissue Cultures studies of the human lymphocytes Science 146 1648 1965

15 Craddock C Nakai G Hajime F and Vanslager L Proliferative activity of the lymphatic tissues as studied with tritium labelled thymidine J Exp Med 121 389 1964

16 Peterson R D Cooper M and Good R A Lymphoid malignancies a new perspective Presented at the 10th Congr of the Europ Soc Haematol Strasbourg (France) 1965

17 Astaldi G Airo R Costa C Duarte N Spleen irradiation and lymphocytes The Lancet i 905 1965

18 Astaldi G Airò R Costa G and Duarte, N Spleen irradiation and immunological *response of peripheral lymphocytes in Chronic Lymphocytic Leukemia* Presented at the 11 Deutschen Hamatologischen Gesellschaft Innsbruck (Austria) 1965 (In Press in Blut)

19 Astaldi G Costa G Airo R and Duarte N Lymphocytes from Leukaemic Blood Cultured with Phytohemagglutinin Europ J Cancer 1 259 1965

a medium containing plasma from normal individuals, gave always low percentages of blasts This means that the performed washings have not changed the usual PHA-unresponsiveness of CLL lymphocytes, and thus that they should be PHA-non responsive because of their innate structure At the same conclusion arrived Schreck (4), Robbins (14), and Oppenheim (12)

The incapacity of the serum from CLL patients to cause inhibition of the blastic development of lymphocytes from healthy persons suggests that the factor responsible for such an inhibition gets lost during the coagulation process, since it is present in the plasma and not in the serum

Studies carried out by one of us (G Costa) have shown, that the inhibiting factor present in the CLL plasma is thermolabile (30 at 56° C), disappears during storage for 2 weeks in the refrigerator at 4° C and that it is destroyed by the X ray irradiation "in vitro" (500 r) Moreover we have been able to show that irradiation of the spleen of CLL patients enables the lymphocytes from these patients to develop blasts after PHA stimulation in a percentage as high as that obtained in culture of lymphocytes from healthy persons (17, 18 19) We have also observed that the plasma of these irradiated patients loses its capacity of inhibiting the PHA-responsiveness of lymphocytes from healthy persons (unpublished data)

In any case the results of these experiments, taken as a whole show that

1) the plasma from CLL patients contains a factor capable of inhibiting the blastic development of lymphocytes from healthy persons, when these lymphocytes are stimulated with PHA in tissue culture,

2) the defective response to PHA shown by CLL lymphocytes can be connected with the structure of these cells and may depend on the fact that these lymphocytes contain (produce?) within their structure the inhibiting plasmatic factor,

3) the factor responsable for such an inhibition gets lost during the coagulation process, since it is present in the plasma and not in the serum,

4) the above mentioned factor is thermolabile (30′ at 56° C), it disappears after 2 weeks at 4° C it is destroyed by the X ray irradiation (500 r) directly delivered "in vitro", and finally it is removed "in vivo" by the spleen irradiation of the CLL patients

Summary

The addition of plasma from chronic lymphocytic leukaemia (CLL) to the PHA culture medium in which lymphocytes from healthy persons are cultured caused the inhibition of the blastic development of these cells On the other hand, the serum from the same CLL patients had no effect in inhibiting the normal lymphocytes in the PHA culture system

The same CLL plasma appeared to be no more inhibiting when kept at 56° C for 30′ minutes and at 4° C for 2 weeks as well as after X rays irradiation in vitro with 500 r

Peripheral blood lymphocytes from CLL patients washed 1—4 times and then cultured in a medium added to plasma from healthy persons did not change their usual hyporesponsiveness to the PHA stimulation in tissue culture

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References

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4 Schrek R and Rabinowitz Y Effect of phytohemagglutinin on rat and normal and leukemic human blood cells Proc Soc exp Biol Med 113 191 1963

5 Quaglino D Cytochemistry in relation to short term tissue culture Proc 9th Congr Europ Soc Haemat (Lisbon 1963) — S Karger Basel/New York Part II/1 p 112 1963

6 Quaglino D and Cowling D C Cytochemical studies on cells from chronic lymphocytic leukaemia and lymphosarcoma cultured with Phytohaemagglutinin Brit J Haemat 10 358 1964

7 Bernard C Geraldes A et Boiron M Action de la phytohémagglutinine in vitro sur les lymphocytes de leucémies lymphoides chroniques Nouv Re Franc Hemat 4 C9 1964

8 Robbins J H Human peripheral blood in tissue culture and the action of phytohemagglutinine Experientia 20 164 1964

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10 Astaldi G Sauli S Airo R Rallo L and Costa G With references to the effect of phytohemagglutinin on lymphocytes from different leukemias Texas Rep Biol Med 23 569 1965

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12 Oppenheim J Whang J and Frei L Immunologic and cytogenetic studies of chronic lymphocytic leukemic cells Blood 26 121 1965

13 Elves W, Stuart R and Israels M Failure of lymphocytes from hypogammaglobulinaemic subjects to transform in culture Brit Med J ii 1051 1964

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From the Departments of Medicine (Head Prof E Ask Upmark M D) and Pathology I (Head Prof G Hultquist M D) University Hospital, Uppsala Sweden

Myelofibrosis Associated with Tuberculous Lymphadenitis

By Sven Mårten Samuelsson, Andreas Killander, Ivar Werner and Björn Stenkvist

Myelofibrosis is a relatively rare disease in this country and nowadays the same holds true also for tuberculosis It was therefore unexpected to find tuberculous infection in four of the ten patients with myelofibrosis treated at this department during the last five years The simultaneous occurrence of tuberculosis and myelofibrosis has been reported in several cases, the majority of which had generalized tuberculosis This led many authors to suggest that tuberculosis was the predisposing factor to the bone marrow changes In our series cervical lymphadenopathy was the first obvious sign of active tuberculous infection In one patient miliary tuberculosis developed later The high incidence of tuberculosis in this series has prompted us to report the findings and to discuss the problems entailed in diagnosis and management

Case reports

Case 1 A H house wife born 1901 One child who had pulmonary tuberculosis at the age of 16 In 1914 the patient had joint pains during one month She was in good health until 1951 when she had a new period of stiffness and pain in the large joints The pains recurred in 1957 at which time a moderate anaemia (Hb 10 g%) was noted In 1959 she was admitted to a hospital for observation There were no joint deformities Hb was 10 g% White cells 1 200—3 000 mm^3 with about 40 % neutrophils and a platelet count between 140 000 and 250 000/mm^3 The sedimentation rate was about 60 mm one hour The sternal marrow showed toxic changes with some features resembling early megaloblastoid degeneration No enlarged lymph glands were found During the hospital stay she had a few attacks of fever which were treated with daily doses of 50—25 mg of cortisone and four blood transfusions She was discharged after 3 weeks and was in good health for the following two years except for slight joint complaints The blood picture however remained unchanged

In July 1961 she began to feel weak tired and hoarse The temperature was about 38° C with some peaks up to 41° C She perspired very much especially at night

An enlarged supraclavicular lymph gland from the right side was removed The histological examination showed a granulomatous tissue consisting of epithelioid cells growing in bands or more diffusely Scattered small fibrinoid or possibly caseous necroses were

seen No ac d fast bac lli or fun o ere found Although the p cture was con pat ble w th *bo h sarco dosis and tuberculos s it* as more n fa our of the former d agnos s X ray of *the lun s sho ed a moderate broaden ng of* th e ned ast num on the r ght s de some sn all alc f cat ons of the rigl t h lar re on and a f w opaci es n tl e left ap cal reg on

In September 1961 her cond t on deter orat ed. Blood exam nat on now sl o ed Hb 62 g% red cells 2.0 mill/mm³ ret culocytes 1 ?— 19% platelets 5000—95000 mm³ and wl te cells 3000—6000 mm³ w th 40—55% mono *nuclears mostly lympho and monocytes* some of them atyp cal a few ery n mature *cells conta n ng one or two nucleol and son e* n elo ytes and erythroblasts Sternal punc ture y elded only blood and a few n arrow cells Both erythropo et c and n yelo d pre ursors sl owed maturation d sturl a ces Tu bercul n sk n test was pos t e for 0 1 mg An nlarged cer cal l mph gland as remo ed Exam na on sl owed abundant mil ar tuberc les w h ep th l o d cells son e g ant cells and w de spread caseous necroses No ac d fast bac lli were found Tl e p cture was compa t ble w th tub r ulous l mphaden t s No tu be cle bac lli ere found n sputun spec mens n d re t n croscop or after guinea p g no ula on Treatment was started w th streptom c n son az d tr amc nolone and blood ansfu ons How er here was no mp o ement dur ng tl e follow ng two mon t s She was transferred to tl e Un ers ty Hosp tal n Uppsala n No ember 1961

On xam nat o a few pea s zed l ard cer cal l mph glands ere noted The l er a d pleen were not palpable Tl e lung f elds e e clear and the r ght s de broade ng of e med ast nun had decreased Tl e sp ne and the pel s appeared ormal on X ray exam nat on Hb as 89% plat lets 20 000 —60 000 mm³ and wh e cells 8 00 mm³ About 80% of tl e leu ocytes ere mono nu l ar w t tl e appearance of blast cells w th nucleoli and a scar e blue cytoplasm. About 50 rythrobla ts per 00 leucocytes ere found Repe ted punctures of the ster un and ac cr st were too hypocellular to perm t ade juate judgu en A surg cal b opsy from the iliac crest re ealed pronounced *myelofibros s* with a few islands of eryth ro and myelopo es s Some megacaryocytes were present Bes des a nun ber of large n ono nuclear cells of ret cular type son et mes w th monstruous nucle were found No tubercles *were observed Tl e subsequent course* as characterized by repeated febr le attacks n creas ng anaem a and *th rombocytopen a wit l* bleed ng tendency No enlarged lymph glands ere noted but the spleen becan e palpable in February 1962 Blood cultures were con tantly negat e Treatn ent w th tuberculosta t cs ant b ot cs cort costero ds a d ell yl testosterone was w tl out effect. The Sab n Feldman dye test for toxoplasmos s was posi ti e n tl e t tre of 1 250 and tl e con plement b nd ng test n 1 240 in the m ddle of Feb ruary Two eeks later tl t tres were 1/1250 and 1 250 *respecti el* Repeated X ray exa m nat ons of the lungs st ll sl o ed clear lung f elds *The tubercul n sens t ty had decreas* ed but a pos t e react on as st ll obta ned w th 3 m g *Streptomyc n and isoniaz d* were d scont nued A new gu nea p g noculat on w th gastr c contents as performed in the m ddle of February The clin cal cond t on deter orated further and the pat ent exp red on March 3 1962 Tl e last gu ea p g nocula t on sl owed no s gns of tuberculos s

Autopsy revealed n l ary tuberculos s af fect ng tl e lungs larynx l er spleen k dneys l mph g ands dura mater and bone marrow Gu nea p g nocu at on th spec mens from he h ar glands gave pos t e rea t on for tuberculos s Tl e same p ture as earl er of pronounced m elof b ros s was found As pre ously noted a small numb r of large ret cular cells ere observed S m lar cells were also found sca ered n tl e lymph nodes a d *tl e spleen*

Case 2 G F lumberer born 1903 In 1955 l e l ad rheumat fe er w th pancard tis and l lateral pleural effus ons After treat n e t h ACTH dur ng one month he re co ered omple ely Tl e tuberculin sk n test was pos t e for 0 1 mg

In Dec. 1961 he not ced swollen cer cal glands on both s des H s appet te was bad

Survey of cases with myelofibrosis and tuberculosis

Author(s) and year	Sex	Age years	Splenomegaly	Hepatomegaly	Enlarged lymph glands	Leucocytes /mm³	Comments	Platelets /mm³	Duration of illness months: Myelofibr	Duration of illness months: Tbc	Tbc type: Miliary	Tbc type: Non miliary organic involvements
Donhauser, 1908	m	58	+++	+	—	11550	no blasts	?	4	?		mesent gl (+fibr pleurisy)
Dyke 1924	m	34	+	?	?	121000—2800	at first considered as myeloid leukaemia	?	6	3	+ (extens bone marrow involv.)	
Krasse & Nothnagel 1925	m	45	++	+	+	27000—4200	myeloblasts 9 % promyelocytes 4 % myelocytes 10 % Myeloid leukaemia?	?	24	48?	+ (wide spread)	
Weil et al 1933	m	58	+++	—	—	22000	no blasts	?	a few months	?		spleen
Hugonot & Soluer 1935	m	64	+++	+++	+	11000	erythroblasts 1 % myelocytes Polycythemic marrow features?	?	26	6?	gl spleen liver	
Stone & Woodman 1938	f	43	++	++	?	94500	myeloblasts 1 % Myelofibr devel 13 years after the diagn of polycyth	128000	>12	?		cerv and hilar gl
Carpenter & Flory 1941	m	33	++	++	+	3100—86300	primitive cells 0—4 % large platelets	+++	40	?	wide spread	
Herr & Frühling 1947	m	40	+	+	—	2000	1 % myeloblasts	37000	< 6	6		lungs and mesent gl
Craig et al 1948	f	39	++	+	+	6000	erythroblasts 2 % myelocytes	138000	18 (156?)	1?	general	
	m	50	++	++	+	3500—11000	5 % myeloblasts normoblasts	251000	18 (20?)	?	liver spleen bone	

	f	30	+ +	++	+	17000—110000	60 % myeloblasts (before death) giant platelets and megakaryocytes	[illegible]			acute	
Migone & Ippolito 1948	f	49	+++	+++	—	3100—12800	no immature cells	440000	36	?	mediast gl + liver	
								?	2	?	acute miliary	
Block & Jacobson 1950	f	28	+++	++		6000						
	f	61	++	—	—	7000	13 % myelocytes	120000	18	120		
Wyatt & Sommers 1950	m	62	++	+	?	7000	?	?	>30	?	*terminal* miliary tbc	
Tsévrénis et al 1951	*m*	30	—	—	+	1900	1 % myeloblasts 4 % myelocytes	?	12 (alive)	12		lungs
Ferzipamuk eu et al 1957	f	63	++	+	+	13000—[illegible]	7 % blasts 8 % myelocytes	?	7 (alive)	7		right lung
André et al 1961	m	67	++	+	+	11000	5 % myelocytes	550000	48 (alive)	?		spleen
	f	52	+++	+	+	21400	1 % promy 1 % myelocytes Myelof devel 3 years after diagn of polycyth	1000000	144 (alive)	6?		cerv gl
	m	6?	++	++	+	17600	probably also polycythaemia	[illegible]00000	14 (alive)	6	lungs + cerv gl	
Anderson et al 1961	?	?	+++	+	?	213000	exposure to ionizing radiat of atomic bomb Terminal tbc	[illegible]9000	50	?	+	
	?	?	+++	+	?	183200		144000	54	?	+	
	?	?	++	—	?	15800	exposure to ionizing radiation of atomic *bomb*	674000	9	?	+	

and he had lost weight In February 1962 he was admitted to the medical department

Physical examination disclosed several firm lymph nodes varying in size from a pea to a plum on both sides of the neck The liver edge was felt about 5 cm below the right costal margin and the spleen just below the left costal margin The temperature was normal

On X ray the lung fields were clear The lumbar spine and the pelvis showed a generally increased density

The tuberculin test (0 1 mg) was positive No acid fast organisms were found in a sputum specimen and gastric contents produced no tuberculosis in the guinea pig Hb was 9 3 g% red cells 3 5 mill/mm³ reticulocytes 2 8 % platelets 440 000/mm³ and white cells 7 200/mm³ A differential count showed 5 % promyelocytes 5 5 % myelocytes 8 % metamyelocytes 19 % band forms 47 % segmented neutrophils no eosinophils 1 % basophils 12 5 % lymphocytes and 2 % monocytes A few nucleated red cells were also found Serum iron was 32 μg% transferrin 173 μg% and haptoglobin 418 mg% (normal value 30—190 mg%) The alkaline and acid phosphatase activity of the serum was normal Vitamin B_{12} in serum was normal

No marrow was obtained on repeated aspirations from the sternum and iliac crest A surgical biopsy from the iliac crest showed myelofibrosis with abundant connective tissue with very few marrow cells Splenic puncture showed numerous erythroblasts as well as some myeloid precursors

Histological examination of a cervical lymph node showed many tubercolous granulomas with central necrosis and epithelioid cells and a few giant cells of Langhans type in the periphery

A diagnosis of cervical gland tuberculosis and myelofibrosis was made The patient was treated with streptomycin for two months followed by isoniazid for one year During this therapy the cervical glands diminished in size In May 1962 the patient was given a series of six injections of 50 mg nortestosterone each resulting in some subjective improvement One month later he was working full time and Hb was 10 4 g% On repeated controls during the following two years there was no significant change in his condition

In July 1964 he had an upper respiratory infection following which he felt increasingly tired weak and dyspneic On readmission in August numerous grape sized hard subcutaneous nodules were palpable all over the body The edge of the liver and spleen extended below the umbilical level Hb was 5 7 g% white cells 6 800/mm³ with about the same distribution as in 1962 Platelets were *85 000/mm³ Sternal marrow was now ob*tained The smear was hypocellular containing mostly erythroblasts and large reticular cells with pronounced polymorphism and many nucleoli The impression of a reticulum cell sarcoma was confirmed by the histological examination of an excised skin tumour Treatment with cytostatics and steroids was ineffective and the patient expired on Sept 28 Autopsy revealed wide spread reticulum cell sarcomatosis osteomyelofibrosis but no signs of active tuberculosis as had been found previously

C a s e 3 A G carpenter born 1889 Since 1956 he had suffered from repeated phlebothromboses in the legs In 1960 splenomegaly was discovered Hb was 12 5 g% white cells 22 000/mm³ A few myeloid precursors and nucleated red cells were seen in the blood smear The platelet count was normal In 1961 a right side renal tumour and ventricular polyposis were diagnosed In August 1962 an enlarged supraclavicular lymph node was observed on the right side and X ray of the lungs showed enlargement of the right hilar region *Microscopically the gland con*tained numerous caseous necroses surrounded by epithelioid cells and giant cells of Langhans type No tubercle bacilli were found in sputum specimens or after guinea pig ino*culations with gastric* contents The patient was treated with streptomycin PAS and isoniazid The blood picture had not changed significantly since 1960

In March 1963 the patient was admitted to the University Hospital On both sides of the neck groups of pea sized lymph nodes were

palpable The liver was felt about 7 cm below the costal margin The spleen reached to the iliac crest Below the liver a grapefruit sized tumour was felt Hb was 14 2 g°/° red cells 5 1 mill/mm³ haematocrit 52—58 °/° reticulocytes 1 2—1 8 °/° and white cells 20 000 —32 0 0 mm³ Differential count myeloblasts 6 °/° promyelocytes 3 5 °/° myelocytes 3 5 °/°, metamyelocytes 2 0 °/° band forms 8 5 °/° segmented neutrophils 61 °/° eosinophils 3 5 °/° basophils 4 0 °/° lymphocytes 6 0 °/° and *monocytes* 2 0 °/° *A few erythroblasts were* found Platelets were 59 000—108,000 mm³ Sedimentation rate 1 mm/one hour Sternal marrow aspiration was difficult A few fragments were obtained which made histological examination possible The marrow was hyperplastic The myelopoiesis — and to a lesser degree the erythropoiesis — showed great activity Many megacaryocytes were seen Streaks of collagen fibres fibroblasts and many vessels were seen indicating early marrow fibrosis Splenic puncture showed myeloid metaplasia Total haemoglobin and blood volume were moderately increased to 911 g (12 3 g kg body weight) and 6 87 litres (91 5 ml kg body weight) respectively The staining of leucocytes for alkaline phosphatase according to Kaplow showed strongly positive granulocytes The heart volume was 830 ml/m² body surface The hilar region was now of ordinary appearance The tuberculin skin test was positive for 1 mg X ray examination of the stomach showed wide spread *polyposis Renal* aortography showed the right renal tumour to be a large cyst The treatment with isoniazid was continued and the patient was discharged in a rather good condition

The patient was later admitted to a hospital for chronic diseases In *September* 1964 *he* died of congestive heart failure Autopsy was performed but adequate histological evaluation was impossible because of advanced postmortal autolysis

Case 4 E F farmer born 1899 A sister had died of pulmonary tuberculosis about 1940 Apart from some periods of manic depressive psychosis the patient was healthy until 1950 when a pronounced splenomegaly was found during a period of *treatment in* the psychiatric clinic. Subsequent investigation at the medical department revealed a spleen extending down to the *iliac crest The* liver was palpable about 7 cm below the costal margin No lymph node enlargement was found X ray films of the lungs showed small apical calcifications Within the whole spine pelvis humeral and femoral bones wide spread areas of osteosclerosis were seen *Hb* was 12 8 g°/° white cells 6 900/mm³ with a *few* myeloid precursors and erythroblasts Platelets were 150 000/mm³ Surgical biopsy from the iliac crest showed typical osteo myelosclerosis with a hypoplastic marrow Splenic puncture showed myeloid metaplasia

After leaving the hospital the patient felt well and did not return for control examination until 1959 After an upper respiratory infection he felt tired lost appetite and complained of perspiration especially at night The spleen extended down to the pubic region Hb was 10 g°/° red cells 3 4 mill/mm³ white cells 2 400—4 400 mm³ and platelets 130 000/mm³ A few myelocytes metamyelocytes and erythroblasts were found in the peripheral blood Without any treatment he improved In 1960 cholecystectomy was performed because of cholelithiasis

In the spring of 1962 he became increasingly tired dyspneic and lost weight There was no fever In August 1962 he was readmitted to the medical department He was now emaciated A few date sized lymph nodes were now palpable on the left side of the neck The liver edge was felt about 9 cm below the costal margin and the spleen filled up the whole abdomen on the left side Hb was 6 7 g°/° red cells 2 6 mill/mm³ white cells 4 200 mm³ and platelets 49 000—94 000 mm³ *The differential count was unchanged* The tuberculin skin test was positive for 1 mg X ray films of the lungs were normal Treatment with steroids methyl testosterone and nortestosterone had no effect. The patient expired in January 1963

Autopsy showed pronounced osteomyelosclerosis and haemopoiesis in liver and spleen On the left side of the neck 5—6 cherry sized firm lymph nodes were found. Microsco

pically they showed tuberculoid caseous necroses surrounded by granulation tissue No other sign of tuberculous infection was noted

Discussion

All four cases showed a histological picture typical for tuberculosis with tubercles with Langhans cells and caseous necroses For some reason no acid fast bacilli were found and unfortunately cultivation or guinea-pig inoculation were not made on autopsy material in three cases The typical histological findings, however, in cases 2, 3 and 4 and the positive result with tuberculostatic treatment in cases 2 and 3 strongly support the diagnosis of tuberculosis

The diagnosis of myelofibrosis seems unequivocal in cases 1 2 and 4 The histological picture of biopsy specimens and autopsy findings were typical The diagnosis in case 3 requires some consideration Obviously his final disease started with the clinical picture of polycythaemia vera At the time of discovery of the tuberculosis the picture was still suggestive of a mild polycythaemia Biopsy specimens of his bone marrow showed marked hyperplasia but at the same time there were streaks of collagen fibres, many fibroblasts and blood vessels, strongly suggesting incipient myelofibrosis

A striking feature in our series is the localization of the tuberculous infection to the lymph glands, predominantly in the neck region Only about 10 % of all cases of active tuberculosis in our area have this localization (10) The explanation might be that the resistance of the lymphoid tissue to tuberculous infection is impaired by the myelofibrotic process

Another possibility is that owing to the effective and thorough public health control by means of regular mass X ray examinations of the lungs in Sweden pulmonary tuberculosis rarely escapes detection and treatment at a very early stage On the other hand tuberculous infection of some lymph nodes might possibly remain undiscovered for many years The occurrence of a debilitating disease, such as myelofibrosis, may activate a latent tuberculous infection A high frequency of tuberculous lymphadenitis would then be expected in our country also in other debilitating conditions As far as we know this is not generally the case

Morrow and Anderson performed an extensive study on active tuberculosis in malignant lymphoma and myelofibrosis in an autopsy material from Hiroshima 1949—1962 (14) No instance of associated active tuberculosis was found in 37 cases of malignant lymphoma despite the fact that the majority of these cases were associated with debilitation and had had a protracted clinical course In contrast to this disseminated tuberculosis was found in association with chronic myelogenous leukaemia (43 cases) and myelofibrosis (12 cases) where the prevalences were 2 5 and 4 5 times respectively that found in the remainder of the autopsy population Thus it seems improbable that the debilitating state per se is the essential mechanism

ehind the occurrence of tuberculosis n myelofibrotic states

The simultaneous occurrence of myelofibrosis and tuberculosis has been reported in several papers The relevant findings of the cases describ d are listed in the table

Some authors have suggested that the myelofibrotic process might be a reaction to the tuberculous infection It is evident that the patients represent a heterogenous group In some cases (1 2 4 5 11 13 18) the myelofibrosis preceded tuberculosis by one or more years The same holds true for case 4 in our series Most of the patients however developed symptoms of both diseases either simultaneously or within a few months Cases 1 and 2 of our series probably belong to this group The possibility cannot be ex cluded however that the moderate anaemia and leukopenia found in case 1 and known two years before the appearance of the tuberculosis might have been the first sign of myelofibrosis As shown in the table there is a great variation in the num ber of leucocytes platelets and imma ture cells in the peripheral blood Thus it may be suggested that the as sociation of myelofibrosis and tuber culosis does not represent an aetiologi cal entity

The simultaneous occurrence of the two diseases is however far too com mon to be merely coincidental This is especially apparent in a community like ours where both conditions are rare Among the explanations offered — aetiological statistical and others — the most probable seems to be that the myelofibrotic disease may dimi nish the resistance to the tuberculous infection

From the practical point of view the coexistence of the two diseases offers certain difficulties in the diagnosis and managements of the patients

The symptoms of tuberculous infec tion may easily be overlooked This is especially true when the pulmonary findings are unobtrusive or missing altogether Symptoms such as fever and chills sensitivity to infections tiredness malaise weight loss mode rate anaemia and granulocytopenia elevated sedimentation rate and the appearance of lymph node and spleen enlargement may all be explained by the myelofibrotic process To judge from our series it is apparently diffi cult to obtain a positive bacteriological diagnosis Microscopic examination of sputum or guinea pig inoculations with gastric contents were constantly negative It must be stressed that his tological examination of biopsy speci mens should always be performed when unexpected lymph node enlarge ment appears in myelofibrosis How ever our experience from the first pa tient (A H) demonstrates that histo logical diagnosis may also be very dif ficult

There is no specific treatment of myelofibrosis *Supportive treatment* with androgens blood transfusions and antibiotics in cases of infection are the principal measures Radiotherapy and busulphan may be used in select ed cases During the last decade cor

tisone or cortisone derivatives have been used more frequently This is especially dangerous in the presence of latent tuberculosis The miliary spread of the tuberculous *infection in* our case I *was most probably* en hanced by *the* discontinuation of the tuberculostatic medication during the corticosteroid treatment In our opi nion even suspicion of tuberculosis in myelofibrosis is a good reason for the institution of long and intensive anti-tuberculous therapy This is especially important when corticosteroids are used

Summary

In a series of ten cases of myelofib-rosis no less than four had active tu berculosis Cervical lymphadenopathy was the first obvious sign of the infec tion Earlier reports of 24 cases with myelofibrosis and tuberculosis are re viewed The possible relationship bet ween the diseases in discussed Both diagnosis and treatment of tubercu-losis may be difficult in myelofibrosis Histological examination should al ways be performed when lymph node enlargement appears Adequate anti-tuberculous treatment should be insti tuted on the slightest suspicion of tu berculous infection in myelofibrosis

References

1 Anderson R E Hoshino T & Yama moto T Myelofibrosis with myeloid me taplasia in survivors of the atomic bomb in Hiroshima Ann intern Med *60* 1 1964

2 Andre J Schwartz R & Dameshek W Tuberculosis and myelosclerosis *with* myeloid metaplasia JAMA *178* 1169 1961

3 Block M & Jacobson L O Myeloid metaplasia JAMA *143* 1390 1950

4 Carpenter G & Flory C M Chronic nonleukemic myelosis Report of a case with megakaryocytic myeloid splenome galy leukoerythroblastic anemia genera *lized* osteosclerosis and myelofibrosis Arch intern Med *67* 489 1941

5 Crail H W Alt H L & Nadler W H Myelofibrosis associated with tuberculo sis A report of four cases Blood *3* 1426 1948

6 Donhauser J L The human spleen as an haematoplastic organ as exemplified in a case of splenomegaly with sclerosis of the bone marrow J Exp Med *10* 559 *1908*

7 Dyke S C Some cases of aleukia or aplastic anaemia associated with throm bopenia Lancet *I* 1048 1924

8 Fevzipamukcu Afetpamukcu Noble T A & Cardon L Myelofibrosis and tu berculosis Quart Bull Northwestern Univ Med School *31* 261 1957

9 Herr R & Fruhling L A propos d un nouveau cas de myélose aleucemique me gacaryocytaire Sem Hop Paris *23* 2519 1947

10 Hillerdal O Tuberkulosens andrade prognos Svensk Lakartidn *60* 1059 1963

11 Hugonot G & Sohier R Splénomegalie myéloide megacaryocytaire amyélocythé mique et tuberculose Sang *9* 933 1935

12 Krasso H & Nothnagel H Atypische Tuberkulose (Geflugeltuberkulose?) bei myeloischer Leukämie Wien Arch f inn Med *11* 507 1925

13 Migone L & Ippolito G Anemia mielo sclerotica ed epatosplenomegalia tuber colare Giorn clin Med *29* 989 1948

14 Morrow L B & Anderson R E Active tuberculosis in leukemia malignant lym phoma and myelofibrosis Arch Path *79* 484 1965

15 Stone D M & Woodman D Polycythaemia terminating in leucoerythroblastic anaemia J Path & Bact 47 327 1938

16 Tsevrénis H Thuillier A C & Panas G Anemie érythroblastique avec osteosclérose chez un tuberculeux pulmonaire Sang 22 78 1951

17 Weil P E Chevallier P & Sée G Splenomegalie myeloide megacaryocytaire amyelocythemique Sang 7 773 1933

18 Wyatt, J P & Sommers S C Chronic marrow failure myelosclerosis and extramedullary hematopoiesis Blood 5 329 1950

Radiumhemmet Stockholm Sweden

Primary Polycythaemia Associated with Multiple Myeloma

By Sixten Franzen, Bo Johansson, Mai Kaigas

During the past fifteen years 293 patients suffering from polycythaemia vera and 132 patients suffering from multiple myeloma have been admitted to Radiumhemmet Among these patients three cases with primary polycythaemia associated with multiple myeloma have been diagnosed This association is very interesting in view of the discussion of a possible relationship between chronic myeloproliferative disorders and plasma cell tumours (1, 2, 4, 5 7 8) Some reports of patients with multiple myeloma and concurrent polycythaemia vera have been published, but only in a few cases have the two diagnoses been well established We therefore, find it of value to report the following three cases

Case 1 (Fig 1) a 63 year old female who was admitted to Radiumhemmet in May 1958 with a 5 year history of weakness shortness of breath warm and sweaty skin and several episodes of spontaneous epistaxis yearly In 1957 her local doctor observed an elevated red blood cell count and an enlarged spleen Her past history was noncontributary

Physical examination revealed a round bodied female with a ruddy complexion The spleen was palpable three fingerbreadths below the left costal margin

Laboratory data The hematocrit value was 51 %, red blood cell count 5 200 000 per cu mm white cell count 9 400 per cu mm platelet count 310 000 per cu mm The sedimentation rate was 6 mm per hour The blood volume was found to be 4 9 liters compared with a calculated normal value of 3 6 liters Total serum proteins were 8 2 gm per 100 ml with albumin 4 0 gm per 100 ml and globulin 2 7 gm per 100 ml Serumelectrophoresis showed a narrow band of abnormal protein in the gamma 1 area Urine was negative for Bence Jones protein

Bone marrow scintigram was performed by injecting collodial Au^{198} intravenously (6) Pronounced uptake was found in both the trunk and the long bones (Fig 2)

X rays of the bones revealed a slight osteoporosis and a slight compression of two vertebrae No local destructive lesions were found

Bone marrow biopsy (sections and smears Fig 3) Only a few fat cells were observed in the marrow fragments The trabeculae were crowded with granulocytopoesis and erythroblasts Huge bizarre megacaryocytes were common and sometimes gathered into groups Some solid proliferations of plasma cells with malignant nucleoli ("myeloma cells") were also observed

Course The patient was treated with 6 mC radioactive phosphorus immediately after admission in 1958 She showed a definite symptomatic improvement All series of blood cell counts decreased in number and the spleen in size In October 1963 she developed

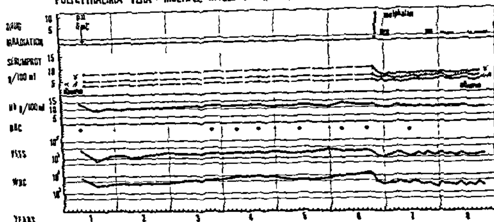


Fig 1 Case 1 The blood cell counts in this diagram are plotted in a semilog scale The dotted areas delinate the normal range of blood counts for the respective components The very slow increase of abnormal globulin during 1958—63 is well illustrated The dark field means total gamma protein values The responses to radioactive phosphorous and melphalan is very striking Note that melphalan is administered in such doses that the blood cell counts follow the lower limit of the normal range of values

a recurring pain in the lower dorsal spine The roentgenograms at that time showed a pronounced compression of two vertebrae She was treated with local radiotherapy and the pains disappeared Treatment with melphalan was also started The patient responded to this treatment with a rather sudden drop in the blood values The abnormal globulin also decreased very rapidly Thereafter the patient received maintenance doses of melphalan Today the patient is symptom free and is able to do her house work All blood cell counts are within the normal range Her serum electrophoresis still shows abnormal globulin but the serum globulin value has decreased to 1 7 gm per 100 ml The roentgenograms show no distinct signs of destructive lesions In the bone marrow no signs of primary polycythaemia can be found but there still exist solid proliferations of malignant plasma cells at about the same extent as before treatment The bone marrow scintigram two years after the start of treatment reveals a less visible uptake of colloidal gold in the marrow of the long bones (Fig 2)

Summary The patient had a marrow picture consistent with the diagnosis of primary polycythaemia and multiple myeloma The symptoms resulted from the polycythaemia which was treated with radioactive phosphorus with good results The myelomatosis was symptom free for more than 5 years When the patient developed symptoms from this disease in 1963 she was treated with a combination of local radiotherapy and melphalan This treatment has to date stopped the progression of the myelomatosis as well as of the primary polycythaemia

Case 2 (Fig 4) a 80 year old male who was admitted to Radiumhemmet because of multiple myeloma in November 1964 The patients history revealed a thrombosis in a leg a few years prior to admission The red blood cell count was normal as late as 1962 at which time he sought medical care for

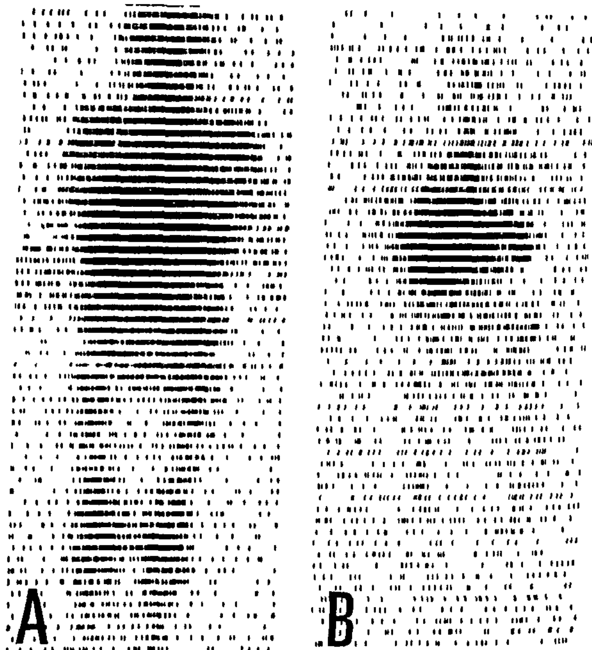


Fig 2 Case 1 Bone marrow scintigrams A Before treatment with melphalan Pronounced uptake of Au^{198} in the marrow of the trunk and long bones *B* Two years after the start of cytostatic treatment The uptake of Au^{198} is less pronounced in the marrow of long bones Because the used technique is not exactly the same in the two scintigrams a direct quantitative comparison is not possible

dizziness He improved on symptomatic treatment After direct questioning he revealed itching after warm bathes In September he fell acutely ill of pneumonia and was hospitalized After treatment with antibiotics he improved rapidly Because of his plethoric appearance and a high red blood cell count a marrow biopsy was performed

Physical examination on admission revealed a highly plethoric patient in good general

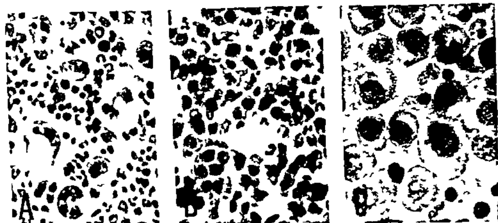


Fig 3 Case 1 Sternal marrow *A* Histologic section of fat free particles with hyperplasia involving all the marrow elements Note the bizarre megacaryocytes This picture characterizes primary polycythaemia (ref 3) Other marrow particles consist of solid proliferations of myeloma cells (*B* histologic section *C* smear)

POLYCYTHAEMIA VERA + MULTIPLE MYELOMA E.L MALE 80 YEARS

DRUGS 100 50 10 5
cytoxan
melphalan
SERUMPROT g/100 ml 15 10 5
albumin
Hb g/100 ml 15 10 5
venesectio
PLTS 10^6 10^5
WBC 10^4 10^3
MONTHS 1 2 3 4 5 6 7 8 9 10 11 12

Fig 4 Case 2 The decrease of abnormal globulin is very gradual Note the different effects of melphalan and cytoxan on the platelet counts The effect on the two diseases of cytostatic drugs is well shown

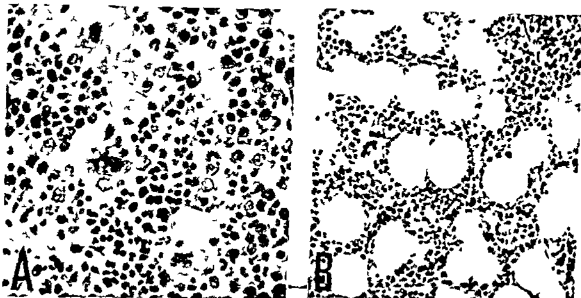


Fig 5 Case 2 *A* Histologic section of fat free *marrow fragments before treatment* The lower left part contains all blood cell precursors Note the *bizarre megacaryocyte* To the right proliferation of malignant plasma cells *B* Sternal marrow seven months after the start of treatment The *fragments* contain normal amount of fat cells No signs of primary polycythaemia *but still* solid proliferations of "myeloma cells" up to the right

condition The spleen and liver were not enlarged

Laboratory data The hematocrit value was 55 % *red blood* cell count 5 900 000 per cu *mm* *white blood cell count 10 600* per cu mm *platelet count 241 600* per cu mm sedimentation rate 0 mm per hour total serum proteins 12 2 gm per 100 ml with albumin 3 7 gm per 100 ml and gammaglobulin 7 1 mg per 100 ml Serum electrophoresis revealed a heavy spot of abnormal globulin in the gamma *1* area There was no Bence Jones protein in the urine The x ray studies of the bones demonstrated some suspicious but no certain destructive lesions of the ribs

Bone marrow biopsy (Fig 5) Fat cells were rare Pronounced hyperplasia of the erythropoesis and granulocytopoesis Megacaryocytes of hyperplastic types were numerous Several solid proliferations of malignant plasma cells were observed

Course Treatment with melphalan was started in a daily dose of 5 mg After an initial fall of all blood values there was a sudden rise of the hemoglobin values The white cell count and *platelet count continued* to decrease At this time the patient felt very ill with severe vertigo as the chief complaint We found it necessary to perform two phlebotomies of 400 ml each The cytostatic treatment was discontinued for a time and *later reinstituted with* doses of *only 2 mg daily* This *lower* dose also produced a *suppression of the platelet count to subnormal* value without any significant decrease of the abnormal protein Therefore we changed *to cytoxan* which was given from April 1965 in doses of 100 mg daily On this course of treatment all blood values returned to and remained within normal limits The abnormal globulin seems *to decrease very gradually* The sedimenation rate that initially was 0 mm per hour increased at the same time the hematocrit value was normalized and has thereafter been 20 to 50 mm per hour The patient is today symptomfree

Summary The patient had a marrow picture consistent with a diagnosis of primary polycythaemia and multiple myeloma Because of the very

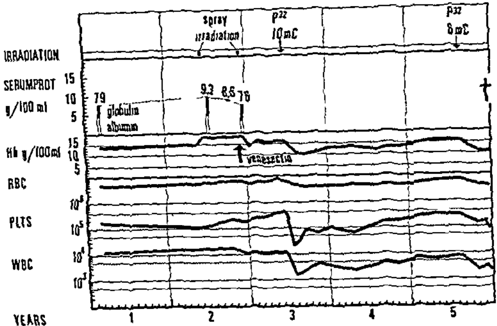


Fig 6 Case 3 This case illustrates the normal response to radioactive phosphorous in a patient with primary polycythaemia Only three estimations of serum protein were performed

high value of abnormal serum protein melphalan treatment was started The effect of this drug on the platelets however was too severe to allow a dose high enough to stop the progression of the myelomatosis Cytoxan therapy was therefore started With this drug it has been possible to obtain a decrease in the abnormal protein Blood values have returned to normal limits The cytostatic therapy has to date made the patient free from signs of primary polycythaemia and also seems to have started a regression of the myelomatosis

Case 3 Fig 6 51 year old female who was admitted to Radiumhemmet in July 1954 because of primary polycythaemia The patients past history revealed that she had enjoyed good health until 1951 at which time she complained of weakness that made her unable to work after October 1952

On admission to her local hospital in June 1953 physical examination revealed a reddish cyanosis of the face She complained of itching in the eyes and her conjunctivae were intensly injected The liver and spleen were enlarged

Laboratory data The hematocrit value was 60 % red blood cell count 5 900 000 per cu mm white blood cell count 14 100 per cu mm platelet count 118 000 per cu mm sedimentation rate 0 mm per hour total serum proteins 9.3 gm per 100 ml with albumin 4 6 gm per 100 ml and globulin 4 7 gm per 100 ml A probable diagnosis of polycythaemia vera was made

Course In June and November 1953 the patient was treated with total body irradiation (spray irradiation) She did not improve

and was admitted to Radiumhemmet in June 1954 On admission the spleen was enlarged The hematocrit value was 59 % white blood cell count 13 400 platelet count 449 000 Bone marrow biopsy revealed no fat cells and a maximal hyperplasia of erythropoesis and granulocytopoesis The enormous number of large megacaryocytes was very striking The number of plasma cells was increased but no solid proliferations were found In June 1954 the patient was treated with 10 mC radioactive phosphorus She improved very rapidly Because of signs of progression in October 1956 she was treated with 6 mC radioactive phosphorus with the same good results In December 1956 the patient complained of pains in the spine radiating down the legs to the toes She related that she had felt similar pains now and then since 1954 The symptoms showed a very progressive course and she developed weakness paresthesias and numbness of the lower limbs In the neurological department a cisternal puncture was performed to localize the lesion by a myelogram Because of a bleeding caused by the examination the patient died At necropsy the examiner beside the mentioned bleeding found an extradural expanding mass in the lower thoracic area The tumour infiltrated the spinal nerves and compressed the spinal cord Similar masses were observed in many vertebrae and in the right femoral bone Other bones were not examined The histological examination revealed the tumour to be a plasmacytosarcoma

Summary The patient had symptoms blood values and a bone marrow picture consistent with the diagnosis of primary polycythaemia After treatment with radioactive phosphorus she improved She suddenly developed symptoms of compression of the spinal cord about two years after the initial diagnosis At necropsy several plasma cell tumours were found in the bones All earlier marrow biopsies have been reexamined No definative signs of multiple myeloma have been found, not even in biopsies taken 6 months prior to death The findings of high globulin values in 1954 and the patients own history of her pains make it most probably that the malignant transformation of the plasma cells had started before this time

Discussion

Patients suffering from two or more malignant tumours derived from different cell lines are of interest from many points of view Especially different tumours belonging to the same organic system in one host will arise questions about etiological factors in malignant tumours Besides the statistical possibility of coincidence, there may be other explanations to such an incidence This may be due to a special constituent of the host making him more succeptable to malignant transformations The possibility of a common unknown agent starting the malignant transformation must also be taken into consideration Among clinicians the possibility that one tumour is the end result of the treatment of the other tumour has been widely discussed Regarding the carcinogenic effects of radioactivity and alkylating agents such a discussion is very natural

In the first two patients discussed here the coincidence of primary polycythaemia and multiple myeloma was diagnosed before any form of antitumour treatment was started This is well illustrated by the marrow biopsies on admission In the third case it

can not be proved that two diseases occurred concurrently but a retrospective study of the case history makes it probable that the myelomatosis had developed before the start of antitumour treatment

We want to underscore the long survival time of case 1 A long survival time is generally accepted as consistent with the diagnosis of primary polycythaemia The general opinion in regards to multiple myeloma is that this desease has a very bad prognosis although long survival times have been reported In case 1 the myelomatosis has shown a very slow progressive course and was symptom free up to five years after the diagnosis In our opinion the prognosis of multiple myeloma today is much better than earlier supposed This is probably partly due to the earlier diagnosis and to the new methods of treatment

Regarding the response to treatment our experience from the three cases shows the well known fact that radioactive phosphorus is very effective in the treatment of primary polycythaemia From these cases it is impossible to draw any conclusions about the effect of radioactive phosphorus on multiple myeloma

The alkylating agents melphalan and cytoxan seem to have effected the course of primary polycythaemia as well as that of myelomatosis Of the two diseases primary polycythaemia has been most effectively improved Although the effect on the myelomatosis is much less pronounced the treatment seems to have been of value to the patients

Summary

Three cases with primary polycythaemia concomitant with multiple myeloma are presented The possible relationship between the two diseases and the results of given treatments are shortly discussed

References

1 Arnholdt F Knotenformige myeomartige Knochenmarkshyperplasien bei Polycythamien Frankf Z Path 1942 57 1—15

2 Brody J I Beizer L H Schwartz, S Multiple Myeloma and the Myeloproliferative Syndromes Am J Med 1964 36 315—319

3 Franzen, S Strenger G Zajicek J Microplanimetric Studies on Megakaryocytes in Chronic Granulocytic Leukaemia and Polycythaemia Vera Acta haemat 1961 26 182—193

4 Giertsen J Chr Polycythaemia Vera with Multiple Myeloma Acta path microbiol scand 1956 38, 439—446

5 Hill G C Polycythaemia and Quadriplegia in Multiple Myeloma Ohio Med J., 1963 59 1005—1008

6 Larsson L G Engstedt L Franzen S Jonsson L Bone Marrow Scintigrams Acta Unio Int Contre le Cancer 1960 16 1473—1477

7 Lawrence J H Rosenthal R L Multiple Myeloma Associated with Polycythaemia Report of four Cases Am J Med Sci., 1949 218 149—154

8 Spickard A Multiple Myeloma with Myelofibrosis and with Polycythaemia Vera Bull John Hopkins Hosp 1960 107 234—240

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Occult Nontropical Sprue and Associated Atrophic Gastritis Simulating Addisonian Pernicious Anemia, With Special Reference to Immunologic Diagnostic Studies

By Paul Brown, M D, Kirk D Wuepper, M D, and H H Fudenberg, M D

Introduction

The differential diagnosis of Addisonian pernicious anemia from other megaloblastic anemias has been facilitated by the use of radioactive vitamin B_{12} uptake tests, serum vitamin B_{12} determinations, and the recently introduced immunological methods for detecting antibody to gastric parietal cells and intrinsic factor and for quantitating intrinsic factor production by the stomach The incidence of antibodies to either parietal cells or intrinsic factor, or both has approached 90 % in patients with uncomplicated Addisonian pernicious anemia (1—3), and 100 % of such patients produce little or no intrinsic factor as measured by immuno assay (4)

The following patient with megaloblastic anemia due to an unusual combination of atrophic gastritis and nontropical sprue illustrates the usefulness of these immunological diagnostic methods and also raises a question of the possible inter relationship of the two diseases

Supported in part by grants from the National Institutes of Health (HE 05997) the Life Insurance Medical Research Fund and the Committee on Research Academic Senate San Francisco Division University of California

Case report

C J (UC # 351780) a 78 year old retired carpenter was born in Denmark and has lived in the United States since the age of 20 As a life long bachelor living alone in small apartments his diet was not above reproach but was not grossly deficient in sources of vitamin B_{12} or folic acid He has never taken alcohol has had no abdominal operations and had not suffered a single serious illness in his entire life In 1963 he was hospitalized for a cataract removal his hematocrit was 32 % and a blood smear showed considerable aniso and poikilocytosis and many macrocytes but the significance of these findings was not appreciated Two months before admission for the present illness in 1965 he noted slowly progressive swelling of the legs and shortness of breath and one month before admission he had mild diarrhea for one to two weeks that subsided spontaneously He walked into the hospital

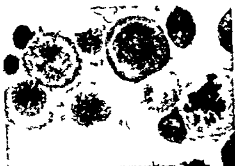


Fig 1 Bone marrow specimen from iliac crest, showing characteristic megaloblastic erythrocytic precursors Wright Giemsa stain Original magnification ×800



Fig 2 Gastric biopsy specimen showing partial atrophy and lymphocytic infiltration Hematoxylin and eosin stain Original magnification ×50

He was an elderly tachypneic and mentally slow man with a pallid lemon colored skin Further examination revealed anasarca bilateral pleural effusions cardiomegaly absent vibratory and diminished positional touch and pain sensation in both legs

A blood smear showed extreme variation in size and shape of the red cells with numerous macrocytes and nucleated red cells target cells hypochromic microcytes and Howell Jolly bodies White cells and platelets were diminished and an occasional hypersegmented polymorphonuclear cell was noted An iliac crest bone marrow was frankly megaloblastic (Figure 1) with almost no stainable iron The white cell count was ...000 platelet count 58,000 red cell count 1 09 million hematocrit 1 5% hemoglobin 3 7 gm and a reticulocyte count was 0 6%

Other studies obtained on admission serum were as follows B... level of 50 μμg/ml normal 150—1,500 folates 8 μg/ml normal 3—2... iron 13... μg% iron binding capacity 2?0 μg% ... % saturation direct total bilirubin 2 1 ... g% haptoglobin 0 LDH 3100 units negative ... test and carotene 0 004 mg% normal 0 05—30

The urinary excretion of radioactive ... in ... hours was 3% normal greater than 10% Repeat Schilling tests with added intrinsic factor produced no effect ... other pa...

tients with pernicious anemia resulted on two occasions in urinary excretion of 1% of the radioactive dose each time Renal function tests were normal Repeat bone marrow showed marked erythrocytic hyperplasia with complete disappearance of megaloblastic cells and a small amount of stainable iron The stomach contained no free acid either before or after stimulation by a standard 50 mg dose of Histalog® Gastrointestinal x rays revealed only a slow gastric emptying time and a dilated small bowel Stools were negative for ova and parasites 10% of the ingested fat was excreted during a 3 day stool collection normal less than 5% and a d xylose uptake test showed 9% urinary excretion in 5 hours normal more than 20%

Antibody to gastric parietal cells was determined in our laboratory according to the method of Taylor and associates (5) Antibody to intrinsic factor and intrinsic factor estimation in gastric juice were determined by Dr Keith Taylor using a micromodification of the method of Ardeman and Chanarin (6) No serum antibodies to either parietal cells or intrinsic factor could be detected, but intrinsic factor was demonstrable in a one hour collection of gastric juice following Histalog® stimulation Biopsy of the stomach confirmed a mild atrophic gastritis, with moderate lymphocytic infiltration and approx



Fig 3 Biopsy specimen of jejunum showing subtotal villous atrophy and lymphocytic infiltration Hematoxylin and eosin stain Original magnification ×50

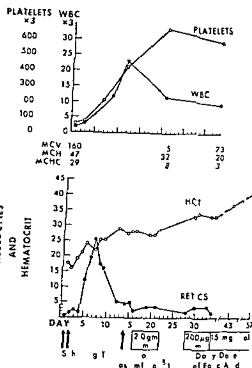


Fig 4 Patient C J Hematological course and pertinent therapy

mately 30 % remaining parietal cells (Figure 2) and biopsy of the jejunum revealed a subtotal villous atrophy (Figure 3)

Addendum After 3 months on a gluten free diet the patient was restudied The serum carotene content had increased from 0 004 to 0 06 mgm% the urinary excretion of D xylose from 9 to 13 % and the fat content of a 3 day stool specimen had decreased from 10 to 7 % The most striking improvement however had occurred in the absorption of vitamin B_{12} a Schilling test (without added intrinsic factor) now showed urinary excretion of 16 % of the radioactive dose in contrast to the previous value of 3 % A gastric biopsy now showed increased mucosal thickness more prominent parietal cells and less inflammation than the previous biopsy Thus improvement in both gastric morphology and function and in intestinal absorption have been clearly documented

Discussion

Initially, many reasons existed for thinking that Addisonian pernicious anemia was the sole cause of this patient s illness The abnormally low urinary excretion of vitamin B_{12} appeared to support this initial diagnosis as did the demonstration of histamine fast achlorhydria

At this point however, three different pieces of evidence made the diagnosis of uncomplicated pernicious anemia untenable First the patient s serum contained no antibodies to either gastric parietal cells or to intrinsic factor, second, a Schilling test with added intrinsic factor produced no increase in vitamin B_{12} excretion and, third serum levels of both vitamin B_{12} *and* folates were low Attention was therefore directed to the possible existence of an unsuspected intestinal disorder and subsequent studies did indeed demonstrate malabsorption associated with biopsy proven nontropical sprue

Meanwhile the patient responded to the Schilling test with a dramatic reticulocytosis gradual hematocrit rise and total elimination of megaloblastic

cells in the bone marrow. Subsequently physiological doses of parenteral folic acid induced a second small reticulocyte response which may have been dampened however, by the earlier large doses of vitamin B_{12}. Could the patient's now well established combined vitamin B_{12} and folic acid deficiency have developed solely on the basis of his small bowel lesion or was gastric disease also playing a part? The finding of achlorhydria had suggested that the stomach was not normal and a biopsy revealed the existence of mild atrophic gastritis *but the contention that this condition* contributed to the vitamin B_{12} deficiency could only be proved by showing the absence of intrinsic factor in the patient's gastric juice and immunoassay documented its presence.

Immunological phenomena in pernicious anemia were at first considered to result from oral treatment with heterologous intrinsic factor (7). Taylor and associates subsequently noted a serum factor capable of combining with and inactivating intrinsic factor in untreated cases of Addisonian pernicious *anemia (5)*. A by *product of* these investigations was the discovery of serum antibodies specific for the gastric parietal cell detectable both by complement fixation and immunofluorescence (8). Antibodies to intrinsic factor are present in approximately 50 % of adults with pernicious anemia (1, 2) and fluorescent antibodies to parietal cells are found in about 90 % of such patients (2, 3, 9). In our laboratory parietal cell fluorescent antibody has been demonstrated in 93 % of patients with uncomplicated Addisonian pernicious anemia.

Antibodies to parietal cells also occur in a small proportion of apparently *healthy people in larger pro*portions of patients with diabetes mellitus and thyroid disease and in about 60 % of patients with atrophic gastritis (2, 9). This information has led Bernhardt and associates to comment that parietal cell antibody fundamentally reflects atrophic gastritis and is not specifically related to pernicious anemia. They concluded that the test, although useful in screening patients *for early asymptomatic atrophic* gastritis is of limited value in the differential diagnosis of anemias (10).

We cannot agree with this conclusion for any test that is positive in nine out of ten patients with pernicious anemia is diagnostically very useful when other conditions often associated with such seropositivity can be excluded. Furthermore a negative result is of even greater significance and should cast serious doubt on the diagnosis of uncomplicated Addisonian pernicious anemia.

Irvine et al. recently demonstrated that the quantity of intrinsic factor in gastric juice from patients with pernicious anemia is clearly less than that in patients with atrophic gastritis who in turn are deficient as compared with normal individuals (4). They suggested that patients at special risk be screened initially for parietal cell antibody and if positive have gastric acid determinations. He further suggested that any histamine stimulated specimen of gastric juice shown to be de

void of acid should be submitted to quantitative intrinsic factor analysis

In the case reported here, our failure to demonstrate antibodies to parietal cells and intrinsic factor was the first clue leading to a re evaluation of the patient's initial diagnosis Because of the subsequent demonstration of vitamin B_{12} malabsorption it was necessary to study the patient's capacity to produce intrinsic factor Like many individuals with atrophic gastritis, however, he secreted only a small amount of gastric juice, and thus immuno assay was the only feasible investigative method Finally having documented by this method the presence of intrinsic factor, and with additional knowledge of the absence of antibodies to parietal cells or intrinsic factor, we are led to speculate that this is *not* a fortuitous association of Addisonian pernicious anemia with nontropical sprue, but rather an instance of atrophic gastritis causally related to the intestinal lesion Wintrobe has stated that intrinsic factor could not be demonstrated in the gastric secretion of a few patients with sprue and that as many as 30 % of patients with sprue have no free gastric acid (11) Our patient is currently being treated with a gluten free diet and will be observed for signs of gastric as well as intestinal improvement

Summary

The unusual coincidence of vitamin B_{12} and folic acid deficiency associated with both nontropical sprue and atrophic gastritis, is reported in an elderly Danish man Although history physical examination and hematological studies suggested a diagnosis of addisonian pernicious anemia the failure to find antibodies to parietal cell antigen led to further investigations, including gastric and jejunal biopsies and estimation of intrinsic factor secretion, which established the primary disease as nontropical sprue The value of immunological methods in distinguishing addisonian pernicious anemia due to a genetically determined deficiency of intrinsic factor from other forms of megaloblastic anemia is emphasized

Acknowledgement

We are indebted to Dr Lionel Head for performing the biopsies on this patient and to Dr Keith Taylor for analyses of serum antibody to intrinsic factor and of intrinsic factor in the gastric juice

References

1 Irvine W J New Eng J Med 273 432 1965
2 Fisher J M & Taylor K B New Eng J Med 272 499 1965
3 Roitt I M Doniach D & Shapland C Ann N Y Acad Sci 124 644 1965
4 Irvine W J Davies S H Haynes R C & Scarth L Lancet ii 397 1965
5 Taylor K B Lancet ii 106 1959
6 Ardeman S & Chanarin I Lancet ii 1350 1963
7 Schwartz M Lancet ii 61 1958
8 Taylor K B Roitt I M Doniach D, Couchman K G & Shapland C Brit Med J ii 1347 1962
9 Adams J F Glen A I M Kennedy L H Mackenzie I L Morrow J M Anderson J R Gray K G & Middleton D G Lancet i 401 1964
10 Bernhardt H Burkett L L Fields M I & Killian J Ann Intern Med 63 635 1965
11 Wintrobe M M Clinical hematology Lea & Febiger Philadelphia 1961 page 502

From The Department for Epileptics (Chief Erik Kiorboe) and the Central Laboratories (Chief Claus Munk Plum) Colony Filadelfia Dianalund Denmark

Megaloblastic Anaemia developing during treatment of Epilepsy

By Erik Kiorboe & Claus Munk Plum

A variety of haematological changes may appear during anticonvulsive therapy For example cases of aplastic anaemia and granulocytopenia have been observed during treatment with *mephenetoin* (NFN) (21 a)

Cases of megaloblastic anaemia have been reported so far unexplained The anticonvulsive drugs suspected as possible causes of megaloblastic anaemia are *phenytoinum* (INN NFN) (1 2 3 4 7 11 17 18 26 29 32 and 34) *primidonum* (INN NFN) (3 4 13 15 29 and 33) *amobarbitalum* (INN) and *secobarbitalum* (INN) (19) *phenobarbital* (INN) (15) and combination of *phenobarbital* and *phenytoin* (20 22) *Barbiturates* are also cited as causing the macrocytosis so often observed during the treatment of epileptic patients The macrocytosis anisocytosis and other haematological features often appear without any signs of anaemia

Thus *Hawkins & Meynell* (18) report that macrocytosis is found in about 45 per cent of patients receiving treatment with phenytoin+phenobarbital in about 34 per cent treated with phenobarbital but only in 25 per cent treated with phenytoin alone

The same authors have examined the vitamin B_{1} content of serum in patients with macrocytosis without anaemia They find that while their controls (22 persons) had a B_{12} content corresponding to 215 $\mu\mu g/ml$ (105—385) a patient group examined had a mean serum content of 175 $\mu\mu g/ml$ Six patients were then given 100 μg of cyanocobalaminum (INN) weekly for a period of two months without any demonstrable change in the blood picture

Folic acid absorption from the intestinal canal and excretion of parenterally given folic acid were normal After the treatment with cyanocobalaminum 20 mg folic acid were given perorally daily resulting in a rapid disappearance of the macrocytosis

Chanarin et all (8) examined the clearance of intravenously injected folic acid in 8 patients with megalo

Review of cases from the literature

	sex	age	Duration of treatment and drugs	Gastric function	Drugs withdrawn	Therapy and effect
Badenoch (1954)	f	22	Phenytoin Na Phenobarbital	achylia		B_{12}
	f	17	Phenytoin Na Phenobarbital	free acid		folic acid
Hawkin & Meynell (1954)	m	52	Phenytoin Na 13 years	free acid		folic acid
Chalmers & Boheimer (1954)	m	32	Phenytoin ca 5 years Phenobarbital ca 5 years Primidonum 2 months			folic acid
	f	47	Phenytoin	achylia		B_{12}
Rhind & Varadi (1954)	f	19	Phenobarbital many years Phenytoin 9 months	free acid		folic acid
Webster (1954)	f	23	Phenytoin 4 years	free acid	+	B_{12}
Berlyne, Levene, McGlashan (1955)	m	28	Phenytoin Phenobarbital 18 months	free acid		folic acid
	m	26	Phenytoin 4 years Phenobarbital 4 years Primidonum 3 months	free acid		B_{12} — no effect folic acid
Ryan & Forshaw (1955)	m	32	Phenytoin 5 years Phenobarbital 5 years	free acid	+	B_{12}
	f	28	Phenytoin 6 years Phenobarbital 8 years	free acid	+	folic acid
	f	27	Phenobarbital ?			B_{12}+folic acid
Vaishnava (1955)	f	28	Phenytoin Phenobarbital 2 years	free acid		folic acid
	f	23	Phenytoin Phenobarbital 2 years ?	free acid		folic acid
Girdwood	f	41	Phenobarbital many years Primidonum ? months	free acid	+	folic acid
[illegible]	[illegible]		[illegible] 17 [illegible]	free [illegible]		[illegible]

[illegible]			[illegible]		free acid	+	folic acid
Fold & Moorhouse (1956)	f	37	Primidonum	4 months	free acid		folic acid
	relapse		Primidonum	7 months	free acid		folic acid
	f	41	Primidonum	4 years	free acid	+	B_{12}
Cyd II (1957)	f	28	Phenytoin	13 years			
			Phenobarbital	13 years	free acid		B_1
	f	26	Phenytoin	2 years			
			Phenobarbital	2 years	free acid	+	Liverextract
	f	30	Phenytoin	7 years	free acid		folic acid
Newman & Summer (1957)	f	57	Primidonum	2 years	free acid	+	B_1
	f	40	Primidonum	1 year	free acid	+	change in food
Christenson Ullmann & Rosemari (1957)	f	48	Primidonum	2 ²/₃ years	free acid		folic acid
	relapse		Primidonum	14 months	free acid	+	folic acid
Forshaw (1957)	f	60	Phenobarbital	1 year			
			Phenytoin	1 year	free acid		folic acid
Volterra & Bonvaldi (1957)	m	33	Phenobarbital	many years			
			Primidonum	5 years	free acid		
Bemans & Hunter (1957)	f	29	Phenobarbital	3 years		+	folic acid
			Phenytoin	3 years			died before treatment
			Primidonum	2 years			
	relapse		Phenobarbital	20 months			
			Phenytoin	20 months	free acid	+	(B_{12})
	m	25	Primidonum	more than 10 months		+	folic acid
			Phenytoin	10 months			
			Phenobarbital	6 months	free acid	+	(B_1) folic acid
	m	51	Phenytoin	4 years			
			Phenobarbital	4 years	free acid		folic acid
Barker (1957)	m	36	Phenobarbital	20 years			
			Phenytoin	4—5 years			
	f	35	Phenobarbital	1 years	free acid		(B_1) folic acid
			Phenytoin	2 years			

Review of cases from the literature

	sex	age	Duration of treatment and drugs		Gastric function	Drugs withdrawn	terapy and effect
Badenoch (1954)	f	22	Phenytoin Na *Phenobarbital*		achylia		B_{12}
	f	17	Phenytoin Na Phenobarbital		free acid		folic acid
Hawkin & Meynell (1954)	m	52	Phenytoin Na	13 years	free acid		folic acid
Chalmers & Boheimer (1954)	m	32	Phenytoin Phenobarbital Primidonum	ca 5 years ca 5 years 2 months			folic acid
	f	47	Phenytoin		achylia		B_{12}
Rhund & Varadi (1954)	f	19	Phenobarbital Phenytoin	many years 9 months	free acid		folic acid
Webster (1954)	f	23	Phenytoin	4 years	free acid	+	B_{12}
Berlyne Levene McGlashan (1955)	m	28	Phenytoin Phenobarbital	18 months	free acid		folic acid
	m	26	Phenytoin *Phenobarbital* Primidonum	4 years 4 years 3 months	free acid		B_{12} — *no effect* folic acid
Ryan & Forshaw (1955)	m	32	Phenytoin Phenobarbital	5 years 5 years	free acid	+	B_{12}
	f	28	Phenytoin Phenobarbital	8 years 8 years	free acid	+	folic acid
	f	27	Phenobarbital	?			B_{12}+folic acid
Vaishnava (1955)	f	28	Phenytoin Phenobarbital	2 years	free acid		folic acid
	f	21	Phenytoin Phenobarbital	2 years ?	free acid		folic acid
Girdwood	f	41	Phenobarbital Primidonum	many years [illegible] months	free acid	+	folic acid

	f	41	Ir I	* y s		-		
(y I I (19)	f	28	I I y I e ol a I l	13 years 17 years		f ee ac l	+	B
	f	26	I I e yl I I of b lal	2 yea s 2 years		f ee ac l		B
	f	33	I I e yl	7 y ars		f ee ac l	+	Li erextra l
New n & Su er (1957)	f	6	I r lo n	? years		fr e ac l		fol c ac d
	f	43	I r n lo u	1 year		free ac d	+	B_1
Cl e so Ull a n & Ro en ari (1957)	f rel pse	48	I r lo u I r n lo u	2*s cars 14 n o ll s		free ac l free ac l	+	cl ange in food fol c ac l
I o sl aw (1957)	f	60	Pl e b rl al I I ylo	1 year 1 year		f ee ac l	+	folic ac l
Vol rra & Ro a ld (1957)	n	33	I I e ol arl t l I e n do	y years 5 years		fr e ac d		fol c c l
Be a s & Hunter (19)	f	5	I I e ol ri al I I e ylo n I r n lo	3 years 3 years y a s		fr e ac l	+	fol c a l I I I f r c t
	relap		I e I rl I I I I	20 o l s 0 o l s				
		2	I r lonu o I e o I enol arl al	ll a 10 10 o l s 6 n o ll s	ll	f c d	+ +	(B) f l e acil
		f1	I I e yl I I e l rl l	4 years 4 yea s		fr e a d	+	(B) f l c ac l
Barker (1957)		30	I I e ol l l I I e ylo	20 y s 4— ye rs		fr e ac l		fol c l
	f	3	I I e l rl I I I I enyl i	l y rs yea s		fr e c l		(B) f l c a l

[1] ( ) ea s o effe t

Review of cases Continued

	sex	age	Duration of treatment and drugs		Gastric function	Drugs with drawn	therapy and effect
Kidd & Mollin (1957)	f	41	Phenobarbital Phenytoin	many years 2 years	free acid		(B_{12}) folic acid
	f	34	Phenobarbital Phenytoin Primidonum	many years 2 years 18 months			died before treatment
B icl er & Ma gold (1957)	f	36	Phenobarbital Mephenetoin Primidonum	18 years 2 years 7 years	free acid		(B_{12}) folic acid
Stokes & Fortune (1958)	m	16	Primidonum Phenytoin	3 years 2 years	free acid	+	folic acid
Gordin (1958)	f	32	Phenytoin Primidonum	many years 6 months	free acid		folic acid
Lees (1961)	f	36	Phenobarbital Phenytoin Primidonum	many years 18 months 18 months	free acid	+	folic acid
	m	34	Phenobarbital Phenytoin	many years 18 years	free acid		(B_{12}) folic acid
	f	46	Phenobarbital Phenytoin Primidonum	many years 14 years	achylia	+ +	(B_{12}) folic acid
	f	34	Phenobarbitone Phenytoin Primidonum	8 years 8 years 4 months	free acid	+ +	(B_{12})
	f	35	Primidonum	1 year	free acid		folic acid
	f	32	Phenytoin	3 years	free acid		(B_{12}) folic acid
	f	31	Phenobarbital	2 years	free acid		(B_{12}) folic acid
	m	5	Phenobarbital Phenytoin	many years many years	free acid	+	(B_{12}) folic acid
K ger (1963)	f	58	Phenobarbital Phenytoin	1 year 1 year	free acid	+	(B_{12}) folic acid (B_{12}) [illegible]

blastic anaemia following or resulting from treatment with anti epileptic agents They found that three of the patients had a normal clearance while the remaining five patients had a very fast clearance The interpretation of the latter result is that the five patients have probably had a reduced folic acid content in their tissues while the three patients have had a normal values of folic acid The authors consider that the origin of the anaemia is an interference with the utilization of folic acid in the tissues

Druskin et al (*10*) report a case of megaloblastic anaemia developing during anti convulsive treatment with a normal diet containing presumably adequate amounts of folic acid If a daily supplement of 25 μ_g of pure folic acid was given the desired effect was obtained The authors interpret this as a sign that the organism is unable to utilize the folic acid ingested in food

In a normal diet folic acid is present in a polymerized bound form as a polyglutamate In the contents of the normal intestine a depolymerizing enzyme is found — conjugase — which is able to convert polyglutamate into free folic acid On the assumption that the anti epileptic drugs administered have an inhibitory effect on this conversion the organism will not be presented with adequate supplies of folic acid This does not occur until pure folic acid is administered

Some investigators consider that this macrocytosis appearing during the treatment of epilepsy is a very early stage of megaloblastic anaemia Among patients with epilepsy the percentage of those who have macrocytosis and subsequently develop megaloblastic anaemia is not known

Christenson et al (*9*) showed that if primidonum was withdrawn in a patient with megaloblastic anaemia remission resulted This must be regarded as support for the argument that primidonum is the direct cause of the anaemia as there has been no lack of folic acid in the tissues

As already mentioned *Hawkins et Meynell* (18) examined the part played by vitamin B_{12} in megaloblastic anaemia They showed that the mean serum content was about *40* $\mu\mu g$/ml below normal *Kidd et al* (21) find values corresponding to 40—50 $\mu\mu g$/ml in two patients while the lowest normal value was 100 $\mu\mu g$/ml

Badenoch (1) *Chalmers* et *Bohetmer* (7) and *Chanarin* (8) quote normal amounts of the vitamin B_{12} According to the literature (1 7 8 21) various vitamin B_{12} tests such as absorption uptake in the liver excretion in the faeces and clearance all appear to give results which lie within normal limits

The studies of *Hawkins* et *Meynell* (*18*) were made on patients on normal diet *Kidd et al* (21) on the other hand found a rise of up to 4 8 % in the reticulocyte count after the ingestion of 100 µg of Vitamin B_{12} *Hawkins et Meynell* (*17*) found a rise of 14 % in the reticulocyte count but no effect on the amount of haemoglobin

There have been very varying results from a number of similar studies It is very difficult to evaluate some of

them, however, as the patient has either had a transfusion (34), or there has been a change in anticonvulsive therapy (27), simultaneously with the vitamin B_{12} treatment

Patients with megaloblastic anaemia resulting from treatment with anti epileptic drugs usually all show free acid in the gastric juice If achylia is found, true pernicious anaemia must be considered to be present

The anaemia usually develops quite slowly Bone marrow studies show a picture which is typical for pernicious anaemia, with a greatly increased number of megaloblasts The myelopoiesis shows a decided shift to the left with asynchronous maturation Poikilocytosis, anisocytosis and a rise in the number of hypersegmented granulocytes are observed in the peripheral blood Serum vitamin B_{12} is reduced in most cases, values down to 40 $\mu\mu$g/ml often being observed The folic acid content on the other hand is usually normal The serum iron is sometimes elevated, sometimes normal, but constitutes up to 60—80 per cent of the transferrin (TIBC) amount

A sudden fall in serum iron may be observed during the treatment with folic acid as early as within 3—4 hours after start of treatment

During the treatment the serum B_{12} most often shows a slow rise A typical reticulocyte crisis is seen even though the means used to combat the epilepsy continue to be employed during the treatment The mechanism of this appearance of megaloblastic anaemia is unknown

Chemically, there is a certain structural resemblance between folic, acid phenytoinum, primidonum and phenobarbital (14) This suggests the possibility of the competitive inhibition of the metabolic process normally associated with folic acid, but it is so far quite inexplicable why such a small proportion of the patients treated with these drugs should develop this type of anaemia

In addition to the literature cited is should be mentioned that Gatenby (13 a) in a restrospective study of 57 pregnancies in epileptics receiving anticonvulsant therapy found no cases of megaloblastic anaemia in 48 pregnancies receiving phenobarbital alone In 7 pregnancies where phenytoin was used as well as phenobarbital there were 4 cases (one unconfirmed) of severe megaloblastic anaemia Folic acid cured the anaemia in these cases although the anticonvulsant therapy was continued Zachau Christiansen et al (35) examinated 367 pregnancies with regard to the amount of folic acid in serum They found decreased values in 31 per cent Among these 367 patients 9 were suffering from epilepsy and in all these 9 the amount of serum folic acid was decreased

Present case

The patient is a 43 years old man who suffered from Huntington s chorea and epilepsy since the age of 28 years

1 st admission Kolonien Filadelfia 26.1 1955 to 3/2 1956 (case record no 34036)

2nd admission Kolonien Filadelfia 1/2 1961 (case record no 41377) for periods staying at a nursing home

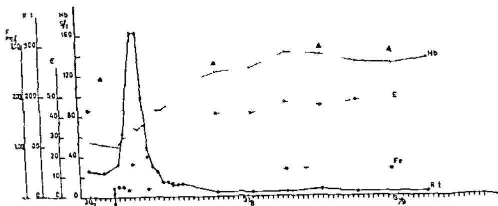


Fig 1 Showing some haematological data during the treatment of a case of megaloblastic anaemia during treatment with anticonvulsiva

● — ● Serum iron
▲ Serum transferrin (TIBC)
• —— • Reticulocytes
○ - - ○ Erytrocytes
× —— × Haemoglobin

Since 1956 the patient has been treated with phenytoinum and primidonum Since 1961 phenytoinum mg 400 primidonum 750 mg Since 1963 chlorpromazinum 100 mg has been given in order to control the choreoid movements During the summer 1965 he developed clinical symptoms of anaemia and a haematological study on the 30 of July showed Hb 55 g l erythrocyte count 1 29 million index of colour 1.37 Erythrocyte volume 180 ml l MCV 140 mpl MCH 306 g/l Chlorpromazinum was withdrawn on the 31/7 and treatment commenced with folic acid mg 10 4 on the 4/8 Chlorpromazinum was readministered from 11/8, and a supplement of iron (Ferromyn Distra) was given from 15 10

The remaining haematological data and the iron concentrations during treatment are seen in fig 1

Gastric juice Free acid (Diagnex blue method) Histamine test meal +Congo reaction Examination of gastric juice for intrinsic factor 1 9) showed positive reaction (We are bringing our thanks to M Schwartz K A S Glostrup for carrying out this analysis) Further data before the beginning of treatment with folic acid Serum folic acid 2/8 0 012 µg ml 6/10 0 075 µg/ml Serum B_{12} 2 8 50 pg/ml 8/9 120 pg/l Serum transaminase normal serum electrolytes normal Thymol and Takata Ara reaction Normal Bilirubin 7 4 mg/ml, serumprotein 59 g/l creatinine 6 mg/l osmotic reaction commencing haemolysis 0 5 °/₀ total haemolysis 0 35 °/₀ NaCl ESR 2 8 21 mm/hr 15/9 2 mm/hr

Iliac crest puncture 2/8 a very cell rich marrow was extracted *Erythropoiesis* completely dominated by megaloblasts and pro erythroblast in all stages of development Only a few normoblast are seen The number of mitoses is greatly increased There are numerous "twin cells *Granulocytopoiesis* numerous "giant metamyelocytes Increased number of mitoses among the myelocytes Pronounced shift to the right Increased number of eosinophilic cells *Lymphocytes* few and normal *Monocytes* few and normal *Plasmacells and haemocytoblasts* increased in number

Diagnosis Observation for pernicious anaemia or related type of anaemia Marrow puncture was repeated after 4 weeks the picture was like that normally found in cases of pernicious anaemia in remission and after 2 months the picture was quite normal

Examination of faeces no positive benzidine reaction no increased fat excretion

After 18 weeks therapy no symptoms of anaemia were present

Discussion

From the history and treatment this appears to be a case of megaloblastic anaemia, adequately treated with folic acid

The investigations carried out would seem to exclude the presence of idiopathic steatorrhoea and chronic disease of the liver It is therefore reasonable to assume that the cause of the megaloblastic anaemia must be sought in the treatment of the epilepsy

Summary

A 43 year old man with Huntington chorea and epilepsy received treatment with phenytoinum and phenobarbital for an extended period The patient has been fatigued for some time, and the laboratory studies revealed a megaloblastic anaemia with nearly normal serum folic acid values The condition was treated with folic acid, without withdrawal of the anti epileptic drugs Normal haemoglobin was obtained and the treatment of the epilepsy must be regarded as the actual etiological factor in the development of the megaloblastic anaemia in this patient

References

1 Badenoch J Royal Soc of Medicine 47 426 1954

2 Barker J C Lancet 2 747 1957

3 Bemians, R C & R A Hunter J Mental Science *103* 606 1957

4 Berlyne M M Levene & A McGlashan Brit Med J 1 1247 1955

5 Berlyne M L A Liversedger & E W Emery Lancet *272* 294 1957

6 Bücher U & R Mangold Schw Med. Wschr 1236 1957

7 Chalmers J N & K Boheimer Lancet 2 920 1954

8 Chanarin I P C Elmers & D L Mollin Brit med J *2* 80 1958

9 Christenson W J E Ultmann & D M Roseman J A M A *163* 940 1957

10 Druskin M S M H Wallin & L Bonagura New Engl J Med *267* 483 1962

11 Forshaw J W B The Postgrad med. J *33* 242 1957

12 Fuld H Brit med *J 1* 1475 1955

13 Fuld H & E H Moorhouse Brit. med. J *1* 1021 1956

13 a Gatenby P B B Lancet *2* 1004 1960

14 Girdwood R H Brit med J *15* 14 1959

15 Girdwood R H & J A R Lenman Brit. med J *1* 146 1956

15 a Gordin R Acta med scand *162* 401 1958

16 Gydell K Acta Haemat *17* 1 1957

17 Hawkins C F & M J Meynell Lancet 2 737 1954

18 Hawkins C F & M J Meynell Quart J of Med *27* 45 1958

19 Hobson Q J G J G Selwyn & D L Mollin Lancet *257* 1079 1956

20 Isager H U f Læger *125* 1655 1963

21 Kidd P & D L Mollin Brit Med J II 974 1957

21 a Kiorboe E & C M Plum U f Læger *116* 47 1954

22 Lees F Quart J of Med 30 231 1961

23 Mollin D L & G I Roos Royal Soc of Med *47* 428 1954

24 Newman M J D & D W Summer Blood *12* 183 1957

25 Peyman M A Brit med J I 1135 1955

26 Rhind E G & Varadi Lancet 2 921 1954

27. Ryan G M S & J W B Forshaw Brit med J 2 242 1955
28. Schilling R F D V Chatanoff & D R. Korst J Lab Clin med 45 926 1955
29. Stokes J B & C Fortune Aust. Ann Med 118 1958
30. Thompson R B Lancet 1 1171 1957
31. Vaishnava H Brit med J 2 791 1955
32. Varadi S Lancet 2 846 1957
33. Volterra M. & G Roumaldi Brit. med. J II 412 1957
34. Webster J M Lancet 2 1017 1957
35. Zachau Christiansen B E Hoff Jørgensen & H P Østergård Kristensen Dan. med Bull 9 157 1962

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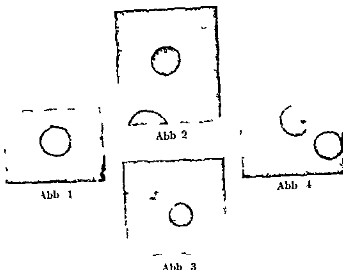
Über das Auftreten basophiler, auf den Erythrozyten aufgelagerter Korpuskel im Blute nach Vornahme einer Splenektomie

Von F Reimann

Im Verlaufe von Untersuchungen über das Auftreten von Howell Jolly schen Körperchen (H J K) im Blute splenektomierter Patienten wurde die Beobachtung gemacht, dass in den fixierten und mit May Grunwald- und Giemsa Losung gefarbten Ausstrichpräparaten neben einer gewissen Anzahl von Erythrozyten mit grossen, tief gefärbten kreisrunden Körperchen im Inneren auch viele andere rote Blutzellen vorkamen, die nur kleine stecknadel kopfgrosse bis punktformige, gut be grenzte und distinkt kolorierte Partikel enthielten Während die grösseren Korpuskel die den H J K entsprachen meist nur in Einzahl vorhanden waren, waren von den kleineren oft zwei, zuweilen drei und nur selten mehr als vier zu zahlen

Bei weiteren Untersuchungen ergab es sich dass alle diese Gebilde sich auch mit basophilen Vitalfarbstoff so vor allem mit Brillantkresylblau dar stellen liessen Sie nahmen diesen Farbstoff sogar recht gierig auf und kontrastierten bei richtiger Farbung durch ihr purpurblaues Kolorit sehr deutlich mit dem gelblichen Ton des Haemoglobins in den intakten Erythrozyten Gleichzeitig zeigte es sich dass die Korpuskel, gross oder klein nichts mit der vitalfärbbaren Substanz der Retikulozyten zu tun hatten und sich von ihr durch das Aussehen und ihren solitaren Charakter unterschieden Sie bildeten stets vereinzelte Gebilde während die retikuläre Substanz den ganzen Erythrozyten durchzog

Die H J K und ihre Eigenschaften sind allgemein bekannt Ähnliche Gebilde wie die kleinen Partikel sind ebenfalls schon von verschiedener Seite beschrieben worden wie aus den Handbuchern der Hämatologie (2) zu ersehen ist Sie differierten aber von den geschilderten Gebilden in einer ganz besonderen Hinsicht Es konnte namlich einwandfrei festgestellt werden dass die beobachteten Partikel sich nicht im Inneren der Erythrozyten befanden sondern stets an der Aussenseite der Erythrozyten an ihrer



Bei allen Abbildungen Phasenmikroskop Öl Immersion ca 1000 fache Endvergrösserung Vitalfarbung mit Brillantkresylblau

Abb 1 Dicht gefärbtes auf den Erythrozyten aufgelagertes Korperchen

Abb 2 Aufgelagertes Korperchen das teilweise uber die Zirkumferenz des Erythrozyten hervorragt

Abb 3 Durch Strange an den Erythrozyten angeheftetes Korperchen

Abb 4 Angelagertes Korperchen durch mechanische Einwirkung des Deckglaschens gegen das Zentrum des Erythrozyten gedrangt wobei die Erythrozytenmembran mitfolgt und eine Eindellung an der roten Zelle erzeugt wird

Wand angelagert waren Dies traf auch fur diejenigen Korpuskel zu die innerhalb der flach ausgebreiteten Erythrozyten zu liegen schienen Diese Position war nur dadurch vorgetauscht dass die wandstandigen Korpuskel in die roten Blutkorperchen projiziert wurden und wegen ihrer Dichte den Eindruck hervorriefen sich in den zentralen Partien der Zelle zu befinden

Beim Studium der Lokalisation der betreffenden Korperchen war das fixierte und mit Giemsa gefarbte Ausstrichpraparat nicht sehr vorteilhaft doch konnte mit Hilfe von schiefer Beleuchtung und bei Anwendung des Phasenkontrastverfahrens bei geeignet situierten Korpuskeln ihre extracellulare Lage deutlich wahrgenommen werden Viel geeigneter erwies sich in dieser Hinsicht die Vitalfarbung da mit ihrer Hilfe auch suspendierte Erythrozyten in noch lebendem Zustand von allen Seiten untersucht werden konnten

Am besten wurde zu diesem Zwecke ein kleiner Tropfen frischen Blutes auf einen

mit Brillantkresylblau gefärbten Objektträger aufgebracht und nach Auflegen eines dünnen Deckgläschens durch vorsichtiges Ausstreichen verteilt. Durch leichtes Andrücken oder Emporheben des Deckgläschens an einer Ecke wurden geringe Strömungen im Präparat hervorgebracht, bei denen die Erythrozyten in Bewegung gerieten, ihre Lage wechselten und beim Zusammenstoss mit anderen roten Blutkörperchen sich um ihre Achse drehten und wendeten.

Schon bei Verwendung der normalen Ölimmersion konnte bei den vital gefärbten Erythrozyten die externe Lage der beobachteten Korpuskel bemerkt werden. Bei den rotierenden und sich wendenden Blutkörperchen konnte recht eindrucksvoll verfolgt werden, wie die anscheinend zentral gelegenen Partikel plötzlich an den Rand des sich drehenden Erythrozyten gerieten und dann deutlich ihre aufgelagerte Position verrieten. Im Phasenbild zeigte es sich sogar häufig, dass die Partikel sich in einiger Entfernung von der äusseren Zirkumferenz der roten Blutkörperchen befanden und mit diesen durch feine Stränge und Fäden verbunden waren. Zuweilen waren sogar nur frei flottierende Fäden sichtbar, an denen kein korpuskulares Gebilde angeheftet war.

Welche Bewandtnis hatte es nun mit den beobachteten basophilen aufgelagerten Körperchen (b. a. K.)? Durch die besondere Lokalisation unterschieden sich die beobachteten Korpuskel von allen endocellulären Gebilden wie den von Heinz und von Schmauch (8) beschriebenen Innenkörpern der Erythrozyten und ebenso auch von den sogenannten Pappenheimer'schen Körperchen (5). Mit den letzteren wiesen sie zwar eine Reihe gleichartiger Eigenschaften auf wie ihr Auftreten nach der Milzextirpation, ihre Kleinheit, die multiple Zahl und die gleichen färberischen Eigenschaften. Pappenheimer und Mitarbeiter (5) bezeichneten sie aber ausdrücklich als endocellulare Elemente (erythrocytic inclusions).

Eine Beziehung zu den siderophilen Granula der Siderozyten war aus dem gleichen Grunde abzulehnen, da auch diese Granula, die ebenfalls nach der Splenektomie in vermehrter Zahl in den Erythrozyten nachzuweisen sind, intracellular gelegen sind. Brüschke (1) stellt in seiner Monographie über den „Siderozyten" die obligate Forderung auf, dass „nur solche Ausstrichpräparate verwendet werden, in denen extracellular keine siderophilen Granuala bzw. Kunstprodukte gefunden werden".

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zu einer Verminderung ihrer Zahl. Im übrigen ist hervorzuheben, dass auch von Pappenheimer und Mitarbeitern bei den von ihnen beschriebenen Körperchen an einen Befall der Erythrozyten mit Bakterien gedacht wurde, eine Ansicht, die sie später wieder fallen liessen.

Die beobachteten aufgelagerten Korpuskel waren also Gebilde von ganz eigener Art. Zum Verständnis ihrer Bedeutung ist zunächst hervorzuheben, dass sie bei Patienten gefunden wurden, bei denen es sich um Fälle von Leberzirrhose und von Pfortaderthrombose mit grossem Milztumor und schwerer portaler *Zytopenie* handelte. Bei keinem der 10 untersuchten Patienten waren trotz sorgfältiger Prüfung die betreffenden Gebilde an den Erythrozyten vor der Splenektomie festzustellen, obwohl in den meisten Fällen eine hochgradige Anämie bestand und im Knochenmark alle Zeichen einer starken regenerativen Hypertrophie und Hyperplasie des erythropoetischen Systems vorhanden waren. Nur nach der Milzentfernung, und dies bereits in wenigen Tagen nach der Operation, waren die Körperchen an den roten Zellen im kreisenden Blut nachzuweisen. Sie nahmen rasch an Häufigkeit zu und waren in einigen Wochen oft fast bei der Hälfte der *zirkulierenden Blutkörperchen zu beobachten*. Später verminderte sich gewöhnlich ihre Zahl und Grösse, doch verschwanden sie nie vollständig aus dem Blut und waren noch viele Jahre nach der Exstirpation der Milz an den roten Blutkörperchen festzustellen.

Die b. a. K. verhielten sich also in dieser Hinsicht wie die H. J. K., die ebenfalls erst nach der Splenektomie im Blut erscheinen und dann durch *viele Jahre nachzuweisen sind*. Auch in anderer Beziehung, wie z. B. in der Färbbarkeit, der Form, liegt eine gewisse Verwandschaft vor. Die H. J. K. können auch in Mehrzahl vorkommen. Nur in der Herkunft scheint ein wesentlicher Unterschied zu bestehen.

Die H. J. K. werden ganz allgemein als Reste des Erythrozytenkernes angesehen. Diese Deutung liess sich aber nur schwer auf die b. a. K. übertragen. Die extracelluläre Lage, das multiple Vorkommen, die Kleinheit und die oft diametral *entgegengesetzte Position* der einzelnen Teilchen an einem Erythrozyten machten eine nukleäre Herkunft der b. a. K. recht wenig wahrscheinlich. Die letzteren Eigenschaften sprachen vielmehr für eine Auflagerung erythrozytenfremder Bestandteile an der Oberfläche der roten Blutkörperchen nach Art eines Coatings. Es konnte sich z. B. vermutungsweise um die Adsorption von Bestandteilen anderer Zellen und Kerne, mit denen die roten Blutkörperchen bei ihrer Zirkulation im Organismus beladen wurden, oder aber auch um die Mitnahme von Resten der interzellularen Substanz aus dem Knochenmark handeln. Für das Vorhandensein eines allgemeinen Belags auf den Erythrozyten sprach auch die erwähnte Beobachtung im Phasenmikroskop von beweglichen fadenförmigen oder strangartigen Auflagerungen an der Oberfläche der Erythrozyten im Blute nach der Milzexstirpation, an denen keine Korpuskel angeheftet waren.

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Travail de la 1ère Clinique medicale de l hopital de la Croix Rouge Hellenique Athenes

La place actuelle dans la nosologie de la Cyanose Methemoglobinemique Hereditaire, "C M H"

Par ANTOINE CODOUNIS

Le but de ce travail est de présenter ici l ensemble des résultats de nos recherches clinicobiologiques et genetiques sur cette curieuse nouvelle maladie genotypique du sang la "Cyanose Methemoglobinemique Hereditaire" de les comparer avec les resultats obtenus par d autres auteurs dans differents pays au cours des 19 dernieres années et de fixer enfin la place definitive qu elle doit occuper dans la nosologie des cyanoses

Par "Methemoglobinemie" on entend certains états morbides dus a l elevation au dessus de la normale du taux de la methemoglobine sanguine et qui cliniquement se manifestent par une cyanose tantot primitive et permanente tantot acquise secondaire et ephemere

La methemoglobine existe normalement dans la proportion de gr 0 10 à 0 15 pour 100 cc³ de sang (Paul) Elle peut cependant s elever dans les différents syndromes cyanotiques jusqu au taux de 32 20 % (Codounis) Elle est toujours intraglobulaire et jamais plasmatique sauf dans les cas des processus hemolytiques infections a anaerobies eclampsie hemoglobinurie paroxystique fievre hemoglobinurique et ictere hemolytique (Bensley) La methemoglobine est un pigment brun different de l hemoblogine en ce que le fer y est a l etat trivalent La methemoglobine (Met Hb) est un veritable oxyde dans lequel un atome d oxygene est uni a un atome de fer C est un pigment inactif qui a perdu sa fonction respiratoire Il s agit d un compose stable dont les elements peuvent etre liberes sous l effet de reactions chimiques La concentration normale de Met Hb se discute encore Elle n est probablement pas superieure a 1 70 % (Wintrobe) de l hemoglobine totale Des que le taux ci haut est depasse une cyanose particuliere apparait

Le sang des sujets ainsi atteints de methemoglobinemie prend une couleur chocolat caracteristique due à la methemoglobine intraglobulaire Le serum par contre conserve sa couleur normale en raison de l intraglobularite du pigment La methemoglobine n est pas toxique mais elle est incapable de

Das vollständige Fehlen der Korpuskel und Auflagerungen auf den roten Zellen vor der Milzentfernung und ihr promptes Auftreten nach der Splenektomie liessen auf die Fähigkeit der Milz schliessen, diese angelagerten „Verunreinigungen" von den Erythrozyten zu entfernen Die starke Deformation der roten Blutkorperchen, die sie bei der Passage durch die Poren der Milzsinus erleiden, wie dies so anschaulich in der Abbildung von Jung (4) demonstriert wird, liess eine mechanische „Waschfunktion" der Milz als durchaus denkbar erscheinen Das bruske Auftreten der aufgelagerten Gebilde nach der Extirpation der Milz und das vollständige Fehlen vor der Operation mache es aber viel wahrscheinlicher, dass die „Reinigungstätigkeit" der Milz schon auch ausserhalb des Organs erfolgt und durch fernwirkende Stoffe im stromenden Blut und Knochenmark bewerkstelligt wird die von der Milz erzeugt und abgegeben werden Die „Sauberung" der roten Blutkorperchen bleibt deswegen prompt und fur die Dauer aus, wenn die Abgabe der „reinigenden" Substanzen, seien sie fermentativer oder anderer Art, versiegt und ihre Quelle mit der operativen Entfernung des Organs fur immer ausgeschaltet wird

Zusammenfassung

Bei 10 Patienten mit Leberzirrhose bzw Pfortaderthrombose, mit Milztumor und portaler Zytopenie waren nach der operativen Entfernung der Milz an vielen Erythrozyten Auflagerungen in Form von runden Korpuskeln und von fadenformigen Strängen zu beobachten, die vor der Splenektomie nicht vorhanden waren Die Korpuskel kamen häufig in Mehrzahl vor, waren kleiner als die Howell Jolly'schen Körperchen und färbten sich mit Giemsa und mit Vitalfarbstoffen wie Brillantkresylblau Sie waren am besten im Phasenmikroskop zu beobachten und waren oft mit Strängen an der Erythrozytenoberfläche angeheftet Die scheinbar zentrale Lage, die sie oft am Erythrozyten aufwiesen, war nur durch die Projektion ins Innere der Zelle hervorgerufen Durch ihre Lage und die färberischen Eigenschaften unterschieden sie sich von anderen körperlichen Gebilden in den Erythrozyten

Es wird nun angenommen, dass es sich um Auflagerungen erythrozytenfremder Substanzen handelt die aus der Zirkulation oder aus dem Knochenmark stammen und die normalerweise bei der Passage durch die Milz entfernt oder durch besondere lytische Stoffe die von der Milz erzeugt werden im Kreislauf abgelöst werden

Literatur

1 Bruschke G Der Siderozyt Akademie Verlag Berlin 1962
2 Heilmeyer L und Begemann H Blut und Blutkrankheiten Handb d inn Medizin 4 Aufl Springer Verlag Berlin Gottingen Heidelberg 1951
3 Jürgens R und Schürer W Schweiz Med Wschrft 75 1055 (1945)
4 Jung F Klin Wschr 36 63 (1958)
5 Pappenheimer A M Thompson W P, Parker D D and Smith K E Quart J Med 14 75 (1945)
6 Röhl H Diss Rostock 1890 cit nach Heilmeyer
7 Schilling V Klin Wschr 6 473 (1928)
8 Schmauch G Virchows Arch 156 201 (1899) cit nach Heilmeyer

sa conna ssance et a son individual sat on ont part c pé d assez nombreux auteurs parmi lesquels le Professeur Codoun s d Athenes et ses collaborateurs Loucatos et Louts des ont certa nen ent apporté la plus grande contri but on Mais en No eml re 1946 Codounis et s s collaborateurs grâce à d importants tra vaux nous rense gnent déf n t ement sur le n ode de transm ss on de la cyanose methé n oglob nén que l éréd ta re rapportant 14 ouveaux cas appartenant a une meme fa n lle de 103 n embres les Vaftoch lari" re part s sur 4 fratries dont 8 branches L etude approfond e de l arbre généalog que de cette fan lle poursui ie par les auteurs malgré de nombreuses d fficultes leur perm t de dé m ntrer pour la prem ere fo s de façon in contestable le caractere héred ta re et fam l al et la nature génotyp que de la malad e Ces cas const tuent des exemples ind scutabl s de transm ss on l eréd ta re de la tare morb de c mme caractere dom nant selon les lo s Men l l e n s

Depu s lors nos conclus o s sur la C M H n ont fa t que recue ll r la conf rmat on d aut res auteurs également comme fa t foi la l ste su ante le cherch urs qui se sont oc cup s de la quest on apr s nous en publ ant les cas olés ou des arbres généalog ques

Ben s Coutlas et Coll 194 K ng et Coll 194 G bson t Harr son 194) Horle n et W ber arbre généalog que complet 1948) Velluz et I ronnet 1948 Luten lacher arbre k n 1949 E ler et Price 1949 Codoun s et l s co le arbre gén de Melan arides 1941 Zac poulos deux arbres gen 1950 Bal za et Sugarn a arbre g n cana l en 1950 Cro zat et oll 1951 Breaks t c ll 1951 Worter Irought et coll 1953 de a per et coll 1953 Becknagel et Horle n a l re ge 1954 Cl iaron et Bonat 1955 ou et F anguelou 1956 Ceral t et coll 55 I c otta et coll 1958 Scott et coll 1958 19 5 1962 Deprée et H ckn an 1955 T w es et Lo ell 1961 T wnes et Mort so 1962 I Mart et c ll 1962 Muller et coll 1963 Betke et Kle nt 1964

I resu é les cas de C M H ent lé n or l le autonon e du globule rouge publ és de pu s 1946 s le e l a 152 y com pr s les 24 a ques de n s deux arbres genéalog ques

Ces cl ffres prouvent que la malad e n est pas aussi rare qu on sembla t le cro re jusqu en 1945 ou on ne conna ssa t en tout que les 21 cas décrits de 1844 à 1944

Si a ces 152 cas on ajoute les 21 cas an ciens qui appart ennent aussi selon nous au cadre de la C M H on arrive au cl ffre de 1 3 que nous a ons pu recueill r dans la l t terature internat onale

On a pu se rendre compte par ce qui pré cède que la Cyanose Methemoglob nem e Héréd taire" att re depu s 1946 l attention de plus en plus cro ssante du monde méd cal Les cas de C.M H publ es sont mult pl és de pu s les comn un cat o s que nous avons fa tes

Beaucoup d auteurs en publ ant des cas isoles ou des arbres généalog ques complets de C M H ont conf rmé de façon incontes tables nos concept ons et nos recherch es Cer ta ns décrivent meme la C M H comme Ma lad e de Codoun s (J Bernard De minas Mu ratore Benhamdoud Wolfgang Kle nt Tow nes etc)

Aujourd hui l ind v dualisat on de la Cya nose meth émoglob ném que héré l ta re co nme maladie autonome l ien déf nie ne la sse plus aucun doute La conv ct on du monde médi cale presque unan me sur nos conceptions et rech erch es concernant cette cur euse maladie héred ta re du glob ule rouge est telle que deux de nos études d ensemble sur la C M H parues dans le Sang 1951 et dans la "Se ma e des Hop taux de Paris 19 2 ont ob tenu un des prem ers pr x a tr bués par le III Congres de l Un on Inter at onale de la Presse M d cale Tor no 19 4) aux me lleurs tra ux or g naux publ és en 1952 et 1953 dans les revues mé l co cl rurg cales apparte nant au groupe de l Un on Internat onale de la Press Méd cale

La place l n tée ne nous p rmettai t pas d entrer ici sur l etude déta llee de la généti que du syndrom e cl n c l olog que etc de l affect n pour lesquelles d a lleurs il y a ac cord unanime de tous les auteurs qui se sont occupes de la ques on nous den anderons au lecteur de se rapporter d une part au trava l presenté au I Congres Internat onal de Généti que Huma ne 195) et l autre part a notre étude générale sur D e Meth amoglol nam en"

fournir de l'oxygène aux tissus Les symptomes de la methemoglobinemie sont *fonction* de l'intensite avec laquelle la methemoglobine remplace l'oxyhemoglobine

A l'heure actuelle deux groupes de cyanoses, dues a la methemoglobinemie, ont retenu l attention d'un tres grand nombre de chercheurs et de cliniciens

1 les cyanoses primitives constitu*tionnelles* stables et parmanentes dans lesquelles les sujets atteints nais*sent* cyanotiques, sans cause apparente Le type representatif de telles cyanoses est la Cyanose Methemoglobinemique Hereditaire" individualisee *par nous* en *1946*, maladie genotypique du globule rouge bien defini, presentant tant le caractere congenital que le caractere familial

2 *les* cyanoses acquises secondaires et ephemeres par intoxication exogene ou endogene dans le cadre desquelles sont comprises les cyanoses medicamenteuses les cyanoses enterogenes et les cyanoses par absorption d'eau de puits tres riche en nitrites

Qu il nous soit permis de ne donner ici qu un resume succinct sur ce qui concerne exclusivement la Cyanose Methemoglobinemique Hereditaire', renvoyant le lecteur en ce qui concerne particulierement la methemoglobinemie acquise pour tous details a notre description generale "Die Methamoglobinamien" parue dans le 3me volume du Handbuch der gesamten Hämatologie, (Urban Schwarzenberg Munchen Berlin 1960 P 710—746) On y trouvera egalement une bibliographie complete sur les cyanoses jusqu en 1956

Historique

L histoire de la maladie est marquee par deux *etapes*

La premiere remonte a 1844 date a laquelle I rançois decrivit le premier cas belge de cyanose sans cardiopathie et se termine un siecle *plus tard* en 1945 avec le remarquable memoire de Sievers et Ryon recueillant dans la *litterature* les seuls 19 cas isolés alors connus y *compris* leur propre cas et les deux cas de Lian et ses collaborateurs

Au cours de cette premiere periode la maladie etait consideree comme se presentant rarement et exceptionellement

Les 21 cas publies jusqu alors (y compris celui de Graybiel (1945) et celui de Barcroft et ses collaborateurs (1945) qui ne figurent pas sur la liste des auteurs precites etaient designes dans la litterature médicale et dans les Traites d Hematologie sous des appelations diverses methémoglobinémie idiopathique methemoglobinemie congenitale ou encore lorsque plusieurs membres d une meme *famille* etaient atteints methemoglobinemie familiale ou encore methémoglobinémie intraglobulaire chronique ou encore enfin cyanose congenitale et familiale etc

La seconde etape commence en 1946 avec la publication de notre premier arbre genealogique de C M H de la famille cyanotique des "Vaftochilari" qui pour la premiere fois a fourni *la preuve formelle de l'hérédité de l'affection* et nous a permis de completer l etude clinicobiologique de cette affection

A Codounis qui la decrit sous le nom de cyanose methemoglobinemique héréditaire lui a depuis en 1946 consacre d importants travaux Il a fixe ses limites nosologiques et reconnu sa transmission" écrivait le Professeur J Bernard (1952) dans son remarquable traite sur les maladies du sang

De son cote Deminas dans sa these (Paris 1951) intitulée "Contribution a l étude de la Cyanose Méthemoglobinemique Hereditaire "Maladie de Codounis" ecrit entre autres "V

Le symptome humoral pathognomonique de cette affection est la présence constante de *la methemoglobine intra cellulaire dans le sang des malades*

Le terme de "Cyanose Methemoglobinemique Hereditaire" que nous avons proposé dès 1946 et qui dès l'abord affirme les trois caracteres de cette anomalie sanguine a) *la cyanose, symptome clinique dominant* b) *la methemoglobinemie caractere humoral spectroscopique pathognomonique* c) *la nature hereditaire de l'affection, dans laquelle nous retrouvons tant le caractere familial que congenital,* est croyons nous le plus indiqué et devrait remplacer definitivement dans la terminologie médicale les appelations de cyanose congenitale ou familiale ou *de cyanose methemoglobinemique* idiopathique intraglobulaire etc qui sement la confusion dans l'etude en general des cyanoses Meme l'appélation "Hereditary Methaemoglobinaemia" qui figure dans certains derniers travaux de la "litterature internationale ne couvre que l'heredité et le signe pathognomonique humoral de la methemoglobinemie Il manque cependant a cette appélation le signe capital clinique qui est "*la cyanose*"

C'est pourquoi nous insistons une fois de plus sur notre opinion que le terme le plus indiqué est celui de la *Cyanose Methemoglobinemique Hereditaire*

Tous les cas qui figurent dans la litterature sous l'une des diverses appelations precitées *ne sont en effet que* des cas de C M H dont les arbres genealogiques n'ont pas été constitués chose evidemment pas toujours facile

Avec la denomination de C M H, le diagnostic differentiel avec les trois autres groupes des cyanoses classiques par anoxemie par melange des sang arteriel et veineux et par polycythemie devient plus aisé étant donne que chez eux *la methemoglobinemie fait defaut*

Le terme de C M H evoque enfin des l'abord dans l'esprit du clinicien le diagnostic différentiel avec les cyanoses du *deuxieme groupe par methemoglobinemie* c à dire les cyanoses *acquises* par intoxication médicamenteuse ou enterogene dans le spectre du sang des patients desquels on trouve soit la bande de methemoglobine soit celle de sulfohemoglobine

Ces cyanoses toutefois sont secondaires ephémeres et intermittentes de guerison immediate aussitot que la cause etiologique disparait (l'intoxication exogene ou endogene)

Bibliographie

Betke and Kleint Hereditary Methaemoglobinaemia (Description de deux familles avec deficience enzymatique Medizinische Akademie Dresden (Mai 1961)

A Codounis Die Methaemoglobinamien Handbuch der Gesamten Hamatologie 2 Aufl Band 3 Urban und Schwarzenberg Munchen Berlin 1960

Depree and Hickman Ann Int Med 51 1078, 1959

Gerald et coll Science 126 300 1957

Gerald and George P Science 129 393 1959

Lockhart et coll Bull et Mémoires Soc. Méd Hôp Paris, 113 877 1962

Muller J et coll Acta Medica Scandinavica vol 173 fasc 2 1963

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Pathogénie

De toutes les hypothèses émises jusqu'ici à ce sujet, il nous semble que deux sont à retenir:

1. celle de Cox et Wendel qui pensent que la reduction de la methémoglobine est, en grande partie du moins, en rapport avec la fonction des enzymes contenues dans les globules rouges ou avec leur absence.

2. celle de Sievers et Ryon basée sur l'absence des systèmes reducteurs des globules rouges.

Nous penchons plutôt pour la première de ces deux hypothèses.

Nous pensons que les magnifiques recherches de Scott et ses collaborateurs, de Townes et ses collaborateurs ainsi que celles de Muller et ses collaborateurs, vont éclaircir à ce sujet bien des points encore obscurs. Ainsi Déficience enzymatique, Diaphorase Méthemoglobine, Hémoglobine M, Déficience du $NADPH_2$ Méthémoglobine Reductase etc ... voilà des chapitres qui sont à l'ordre du jour, afin d'élucider le problème si passionnant de la pathogénie de la Méthémoglobinémie Heréditaire.

Quoi qu'il en soit, le fait essentiel demeure que la maladie est hereditaire et que le globule rouge acquiert par heredite la tare morbide, le rendant inapte à réduire la méthémoglobine en hemoglobine.

Conclusions

Il ne fait point de doute, après nos propres recherches suivies de celles d'autres auteurs au cours de ces dernières 19 années, que la "Cyanose Méthémoglobinémique Héréditaire" (C.M.H.) *constitue dans la nosologie des cyanoses une entité morbide du globule rouge, autonome et bien definie.*

Certains auteurs ont bien voulu donner à cette entité morbide le nom de 'Maladie de Codounis", "Codounis Disease", "Codounis'sche Krankheit", "Morbus Codounis".

Dans cette entité entrent toutes les cyanoses constitutionnelles, congenitales, familiales et héréditaires. Ainsi aux trois groupes classiques de cyanoses par anoxémie, par melange des sangs arteriel et veineux, par polycythemie, il convient d'en ajouter un quatrième, celui de la *"Cyanose Méthemoglobinémique Héréditaire (C.M.H.)"* maladie se transmettant héréditairement au globule rouge et le rendant incapable de réduire la méthémoglobine en hémoglobine, cyanose primitive et permanent.

Cette maladie enzymatique, tout comme la maladie hémolytique congénitale, se transmet sans prédilection tantôt par les femmes, tantot par les hommes, en predominance toutefois par les hommes et plutot selon le caractère dominant que recessif.

Bien que sur les 173 cas environ actuellement connus plus de 50 soient de provenance hellénique, la maladie n'est pas particulièrement grecque. *Rependue dans tous les pays, elle n'est pas aussi rare qu'on le supposait.*

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Depree and Hickman Ann Int Med 51 10 8, 1959

Gerald et coll Science 126 300 1957

Gerald and George P Science 129 393 1959

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Nous penchons plutôt pour la premiere de ces deux hypotheses

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IV

ENDOCRINE SECTION

Pisciotta J Lab and clin Med 54 78, 73—87, 1959
Scott and Hoskins Blood 13 794 1958
Scott and Griffith Biochimie et Biophys Acta 34 584 589, 1959
Scott E M J of Clinical Investigation 329 1960
Scott E M and Mc Graw J C Journal of Biol Chemistry Vol 237 no 7 (1962)
Scott E Biochem and Biophysical Research communications 9 59—62 1962
Townes P L and Lowell G B Blood 18 18, 1961
Townes and Morrison Blood 19 60 1962

Medical Unit St Mary's Hospital London.

Carcinoid Tumours

By W S Peart M D F R C P

One of the outstanding examples of the combination of clinical acumen and biochemical knowledge shown by Jan Waldenström has been the elucidation of the carcinoid syndrome Following the early description by Cassidy (3 4 5) of the flushing in two patients one said to have a carcinoma of the stomach the description of the clinical picture with right sided valvular lesions in the heart diarrhoea and flushing of various types was developed in studies by Waldenström and Ljungberg (33 34) Thorson and his colleagues (30) and Biorck Axen and Thorson (2) When Lembeck (13) found large quantities of 5 hydroxytryptamine (5 HT) in the tumours it was not long before Pernow and Waldenström (22) *demonstrated excessive amounts of* 5 HT in the blood and urine of such cases Later it was found that most of the 5 HT even in these patients was bound in the platelets One of the final excretion products 5 hydroxyindole acetic acid was demonstrated to be increased in such patients by the work of Page and his colleagues (16) and Sjoerdsma and Udenfriend (26) The direct proof that the tumour and its products were responsible for the clinical manifestations was best shown in a case reported by Thorson and his colleagues (31) where such a tumour developed in a teratoma of *the ovary and removal was followed* by a remission At this time since much knowledge about the action of 5 HT had accumulated from the work of Erspamer (6) who had extracted enteramine from the intestine wall and had correlated it with the pre*sence of the cells of Kultschitsky* it was natural to attribute most of the symptoms and pathological changes to an excess of the active substance 5 HT The demonstration that intravenous 5 HT could cause facial flushing both in normal subjects and in *patients with carcinoid* disease (17) added further weight to this view Another twist to the biochemical story came when Waldenström Pernow and Silwer (35) showed increased urinary excretion of histamine in many patients They discussed the *part that it might play* in the syndrome and in some of their patients who had a peculiar geographical red

tially that some humoral influence attacked the endothelium and was removed in passage through the lungs it must not be forgotten that the left side may be affected in some patients (29) All efforts to convincingly demonstrate that 5 HT can produce such valvular disease on administration to experimental animals have failed The histology of the valve shows the process of damage followed by repair and it would equally be arguable that an enzyme which attacked endothelial collagen could bring about this effect The change is not peculiar to the valves and occurs in the lining of the atrium and ventricle as well as in pulmonary arterioles

Flushing

This most prominent feature of the syndrome was of course earlier thought to be due to release of 5 HT but our own investigations (18 21 24) cast considerable doubt on this The first observation is that the typical spontaneous flush is most commonly bright orange to red and is a hot flush This type is almost impossible to reproduce by a wide range of doses of 5 HT in patients with carcinoid disease Our studies on the precipitation of flushes using small doses of adrenaline and noradrenaline showed that the most likely explanation of this flush which was exactly like the spontaneous flush was the release from the tumour and its metastases of a substance or substances which caused vasodilatation In this type of patient with a red warm flush direct examination of blood draining metastases did not show an increase in the amount of 5 HT in the plasma Another strong point was that administration of 5 HT to these patients led to increased respiration and in some to marked dyspnoea which in most of these patients was absent even during the deepest flushes However other types of patient were observed with blue to red flushes and a cooler skin who became wheezy during their flushing attacks Direct examination of blood draining from this tumour revealed increased amounts of 5 HT in the blood during the attack (1) so that while this type of patient undoubtedly releases 5 HT which causes some of the symptoms the other type probably does not At that time we felt for various reasons that histamine was unlikely to be directly implicated Recently Oates and his colleagues (15) have shown increased amounts of bradykinin in the blood draining the tumour in various patients in whom a flush had been provoked by adrenaline or noradrenaline They further extracted a kallikreinlike enzyme from the tumour tissue Bradykinin intravenously causes a marked red warm flush and also lowers the blood pressure in many such patients In many of the patients we studied the blood pressure was lowered during a flush but by no means always and the most reasonable explanation for the flush now seems to be bradykinin or a very similar substance

*

flush with itching, it was suggested that there was either a general or local release of histamine. As far as the biochemical pathways in the tumour are concerned, apart from the presence of large amounts of 5 HT and the decarboxylase which seems to be a very universal enzyme and relatively non specific (32), Grahame Smith (8) has more recently demonstrated the enzyme which hydroxylates tryptophan in the tumour and has described some of its co factor requirements. Demonstrating how the study of a tumour can yield variable information bearing on normal physiology, he has shown the presence of this enzyme in those regions of the brain which are known to contain large amounts of 5-HT (9, 10).

It would now be well to turn to a critical examination of the relation between symptoms biochemistry and pharmacology in this condition. From the work of Erspamer and others (6), it was known that 5 HT contracted many smooth muscles and is of course responsible for part of the vasoconstrictor action (serotonin) in serum. This had previously led to the extraction of 5 HT from serum by Rapport Green and Page (23).

Diarrhoea

The stimulant action of 5 HT on intestinal smooth muscle led naturally to the belief that this was the cause of the severe diarrhoea and increased intestinal motility noted in these patients. This may well be so especially since the diarrhoea is one of the few manifestations which can be abolish by use of 5 HT antagonists (20).

Oedema

Oedema is more difficult to unde stand, particularly the type which o curs on the face and hands during s vere flushing episodes. It seems ve unlikely to be due to 5 HT and som substance which increases capillar permeability must still be sought fo

Skin changes

Apart from the flushing, the nex commonest skin manifestation is th permanent telangiectases, and the have not been satisfactorily explained. A most interesting patient was firs described by Thorson and his colle agues (31) in whom pellagrinous ski changes were reversed by the admi nistration of niacin. There is a strong suggestion that some endogenous tryptophan is converted to niacin in the body and in an ill patient with diarrhoea and a poor intake of food the endogenous source might be of prime importance. The avidity of tumour cells for tryptophan might then cause deficiency of niacin and pellagra would result. The more general idea that tumour cells can successfully compete with other cells of the body for essential metabolites is of great importance in considering the metabolic effects of other cancers.

Valvular heart disease

While the high incidence of right sided valvular disease suggested in

ginally have been biochemically toti potent, the process of becoming cancerous involves the preferential release of one of these originally possessed pathways It would carry the implication that these pathways are only being suppressed and given the right conditions can be revealed In considering the mechanisms behind differentiation of cells gene suppression is considered to be of great importance (12 14) leaving the other gene controlled enzyme systems to develop preferentially Imaginative study of these sorts of cancer cells may help to throw some light on this process An alternative hypothesis which is to me much less attractive is that by chance when cancer affects a cell the biochemical derangement produced leads to the production every now and again of a pharmacologically active substance In the first place the precise structure of some of these pharmacologically active substances in these tumours may shed light since obviously if for example the "vasopressin type" molecule produced by a tumour of the lung is different in some respect from the naturally occurring vasopressin some alteration in the ordinary biochemical pathway must be admitted and the first hypothesis will not stand on its own

It can be seen from this line of thinking and work how much we owe to the original observations of Jan Waldenstrom

References

1 Andrews T M Peart W S & Robertson J I S J Physiol *155* 8P 1960

2 Biorck G Axén O & Thorson Å Amer Heart J *44* 143 1952

3 Cassidy M Proc R Soc Med 24 140 1930

4 Cassidy M Proc. R Soc Med 24 920 1931

5 Cassidy M Proc R Soc Med 27 220 1934

6 Erspamer V Arch int Pharmacodyn *93* 293 1953

7 Euler U S von Noradrenaline Springfield, Ill Charles C. Thomas 1956

8 Grahame Smith D G Biochim biophys Acta 86 176 1964

9 Grahame Smith D G Biochem biophys Res Commun *16* 586 1964

10 Grahame Smith D G & Moloney L Biochem J 96 66P 1965

11 Harrison M T Montgomery D A D, Ramsey A S Robertson J H & Welbourn, R B Lancet *1* 23 1957

12 Jacob F J Molec Biol *3* 318 1961

13 Lembeck F Nature *172* 910 1953

14 Monod J J Molec Biol *6* 306 1963

15 Oates, J A Melmon K Sjoerdsma A Gillespie L & Mason D T Lancet *1* 514 1964

16 *Page, I H Corcoran A C* Udenfriend S Sjoerdsma A & Weissbach H Lancet *1* 198 1955

17 Page I H & McCubbin J W Circulation Res *1* 354 1953

18 Peart W S Andrews T M & Robertson J I S Lancet *1* 577 1961

19 Peart W S Porter K A Robertson J I S Sandler M & Baldock, E Lancet *1* 239 1963

20 Peart, W S & Robertson J I S Lancet 2 1172 1961

21 Peart, W S Robertson J I S & Andrews T M Lancet 2 715 1959

22 Pernow B & Waldenstrom J Lancet *2* 951 1954

23 Rapport M M Green A A & Page I H J biol. Chem *174* 735 1948

A number of patients have been observed in recent years who have thrown further light on this syndrome The biochemical aspects were first emphasised by Sandler and Snow (25) and the characteristic is that large amounts of 5-hydroxytryptophan are excreted in the urine Normally it seems likely that the tumour cells contain enough oxidative enzymes to carry out the conversion of most of the 5 HT produced to 5 hydroxyindole acetic acid, so that only small amounts of 5 HT reach the urine and practically no 5 hydroxytryptophan In this sort of patient, however, large amounts of both are present in urine This suggests that the tumour cells are deficient in oxidative enzymes so that 5 hydroxytryptophan is released into the circulation and appears in the urine or is converted to 5 HT, which then itself appears in the urine Williams and Sandler (37) pointed out that derivatives of the primitive fore gut like the pancreas bile ducts and bronchus give rise to this biochemical type of tumour The absence of an enzymatic pathway is common in many cancers It seems likely that the predominance of noradrenaline producing phaeochromocytomas is due to a *deficiency of the methylating enzyme* converting noradrenaline to adrenaline Again such tumours are known which produce hydroxytyramine or even dopamine (7) In the case of the thyroid cancer cells seem incapable in most instances of iodinating the tyrosine fully so that the precursors mono and diiodotyrosine are produced (27 28) In some tumours it may therefore be a general rule that the final biochemical step is the only one which is removed and this raises the possibility that there is a strong association between cancer properties and a fairly orderly removal of a small part of the cell s biochemical potential The relation between these two observations needs a lot more study Another important point raised by these tumours is that, as in a patient studied at St Mary s Hospital (19), the primary tumour appeared as a pancreatic duct carcinoma and did not look like a conventional carcinoid tumour Does this mean that a cell with a different histological appearance is really a modified carcinoid cell of the same embryological origin as the cells of Kultschitsky? This might well be the case in some tumours which have the same histological appearance as typical carcinoid tumours e g those occurring in the lung but many are described which are indistinguishable from cancers of particular organs such as oat cell carcinoma of the lung (11 36) This brings the carcinoid syndrome into relation with all the other biochemical and pharmacological syndromes described in relation to cancers *in different organs e g* Cushing s syndrome in association with lung cancer ADH secretion with lung cancer and phaeochromocytoma in association with thyroid carcinoma, to mention but a few The nature of this association has emerged as one of the most fascinating problems in cancer The most impressive hypothesis would be that since all cells must ori

From the University Department of Pathology Allmanna Sjukhuset Malmo Sweden

On the Prevalence and Incidence of Carcinoids in Malmo

By Folke Linell & Kerstin Månsson

Various authors have studied the incidence of carcinoid (1—7) Some of the figures given vary widely This discrepancy may be due to differences in the composition of their series i e heterogenous or selected or to the use of different or unsatisfactory examination methods It was therefore considered legitimate to present an analysis of the frequency of carcinoids found at biopsy and necropsy in the rather well defined population of Malmo which as Waldenström (8) points out is very well suited for frequency studies

Material and methods

The material derives from the town of Malmo which has about 230 000 inhabitants The town has only one general hospital which serves the entire population of the town The necropsy frequency at the hospital is high (98—99 %) and about 60 % of all persons dying in the town are examined post mortem The necropsies were performed at one department och with uniform methods The gastrointestinal tract was always slit up rinsed and carefully examined In cases with tumours a special search was made for metastases and in all such cases specimens were examined microscopically The prevalence (defined according to recommendations of WHO "as the frequency of illnesses *in existence* during a defined period whether they started before or during the period") was studied in necropsy material from the years 1959—1962

The incidence (defined according to recommendation of WHO as "the frequency of illnesses *commencing* during a defined period or of persons becoming ill during the period) was studied in biopsy material from the years 1957—1962 Since all inhabitants of the town can receive hospital care free of charge it may be assumed that practically all cases of carcinoid that produced symptoms or that were *discovered incidentally at operation* are included in the present *material* The possibility of the disease having been discovered and treated surgically in some other hospital is negligible and can hardly influence the present analysis

24 Robertson J I S Pearl W S & Andrews T M Quart J Med *31* 103 1962
25 Sandler M & Snow P J D Lancet *1* 137, 1958
26 Sjoerdsma A & Udenfriend S J clin Invest *34* 914 1955
27 Stanley P G Biochem J *63* 581 1956
28 Tata J R Rall J E & Rawson R W J clin Endocrin *16* 1554 1956
29 Thorson Å H Acta med scand Suppl 334 1958
30 Thorson, Å H Biorck G Bjorkman G & Waldenstrom J Amer Heart J *47* 795 1954
31 Thorson Å H Hanson A Pernow B Söderström N Waldenström J Winblad S & Wulff H B Acta med scand *161*, 495 1958
32 Udenfriend S Proc. joint W H O — Czechoslovak Cardiol Soc Symposium on the Pathogenesis of Essential Hypertension Prague p 351 1960
33 Waldenstrom J & Ljungberg E Sv läkartidn *50* 690, 1953
34 Waldenström J & Ljungberg E Acta med scand *152* 293 1955
35 Waldenstrom J Pernow B & Silwer H Acta med scand *156* 73 1956
36 Williams E D & Azzopardi J G Thorax *15* 30 1960
37 Williams E D & Sandler M Lancet *1* 238 1963

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31 Thorson Å H Hanson A Pernow B Soderström, N Waldenstrom J Winblad S & Wulff H B Acta med scand *161* 495 1958
32 Udenfriend S Proc. joint W H.O — Czechoslovak Cardiol Soc Symposium on the Pathogenesis of Essential Hypertension Prague p 351 1960
33 Waldenström J & Ljungberg E Sv läkartidn *50* 690 1953
34 Waldenstrom J & Ljungberg E Acta med scand *152* 293 1955
35 Waldenstrom J Pernow B & Silwer H Acta med scand *156* 73 1956
36 Williams E D & Azzopardi J G Thorax *15* 30 1960
37 Williams E D & Sandler M Lancet *1* 238 1963

Table 4 Metastases in carcinoids of gastrointestinal tract (14 cases)

Regional lymph nodes in mesenterium	10 cases
Other lymph nodes	4
Liver	7 "
Spleen	1 "
Peritoneum	1
Skeleton	2 "
Adrenal	1 "
Pleura	1
Colon	1 "
Heart	1 "
Pancreas	1
Kidney	1
Lung	1
Thyroid	1

been performed 3 years previously for carcinoid of the caecum and according to the hospital records a typical carcinoid syndrome had been manifest before and had persisted after the operation In three other cases carcinoids were found incidentally at operation Diffuse abdominal pain had been noted in 2 cases and might have been due to carcinoid In addition in 2 cases with extensive carcinoid growth symptoms of intestinal cancer had been observed HIIA examinations had of course not been performed in cases in which carcinoid *had not been suspected*

In only 4 of the 53 cases could the carcinoid be regarded as the main cause of death In all the other cases the carcinoid was an incidental finding In 40 % of the cases there was also some other malignant tumour a frequency corresponding to that of malignant tumours in the entire necropsy material

Biopsy material This material dates from a six year period (1957—1962)

Table 5 Gastrointestinal carcinoid (operated series)

Sex	Age (yr)	Symptoms or main disease	Localisation of carcinoid
♀	80	Gastric symptoms	Stomach
♂	60	Colon cancer	Small intestine
♂	68	Colon cancer	Small intestine
♂	38	Symptoms of appendicitis	Small intestine
♂	65	Abdominal pain	Appendix
♀	40	Abdominal pain Ovarian endometriosis	Appendix
♀	40	Abdominal pain, Myoma of uterus	Appendix
♀	23	Symptoms of appendicitis	Appendix
♀	34	Symptoms of appendicitis	Appendix
♀	15	Symptoms of appendicitis	Appendix
♂	57	Rectal polyps	Rectum
♀	60	Sigmoid polyp Rtg changes	Sigmoid

There were all together 12 cases of gastrointestinal carcinoid (see Table 5) including as many as 6 cases of carcinoid of the appendix and 3 of the small intestine One case was gastric cancer of carcinoid type In another case a large tumour (14×10 cm) was found in one of the ovaries The tumour was a teratoma with only carcinoid structure in the abundant connective tissue This was the only tumour that had produced a carcinoid syndrome with flush The symptoms caesed after operation when the HIIA values also became normal In *the 12 cases with tumours of the* gastrointestinal tract carcinoid may be regarded as the indication for opera

Results

Necropsy material The prevalence of carcinoid is given in Table 1 All together 53 cases of gastrointestinal carcinoid were seen, which means a prevalence of 1 1 % The frequency of bronchial carcinoid was only 0 1 %

The age and sex distribution is given in Table 2 Carcinoid was never

Table 1 Prevalence of carcinoids in autopsy material

Year	Necropsies	Carcinoids	
		Gastro intestinal	Bronchial
1959	1094	7	0
1960	1194	12	3
1961	1220	15	1
1962	1320	19	1
Total	4828	53	5

Table 2 Age distribution of carcinoids in autopsy material

Age	Carcinoids			
	Gastrointestinal		Bronchial	
	♂	♀	♂	♀
40—49	2	—	—	—
50—59	—	2	—	1
60—69	18	7	—	—
70—79	10	2	—	2
80	9	3	1	1
Total	39	14	1	4

Table 3 Site of carcinoid (autopsy material)

Oesophagus+stomach	3
Duodenum	3
Jejunoileum	43
Meckels diverticle	1
Appendix	—
Caecum	3
Colon rectum	—
Total	53

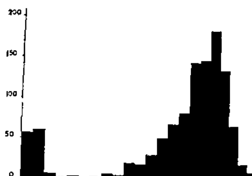


Fig 1 Age distribution of autopsy material The two first columns are perinatal mortality the following are 5 year classes

found below the age of 40 years The necropsy material, however, belonged to higher age classes, as is apparent from Fig 1, which shows the age distribution for the year 1959 This distribution is representative of the entire material

The sites of the gastrointestinal carcinoids are given in Table 3 Carcinoids of the small intestine were most common and were seen in 43 of the 53 cases The abscence of carcinoid of the appendix is noteworthy About twenty-five per cent (13 of 53) of the carcinoids were multiple In these cases the number of tumours varied between 2 and 14 Sometimes the carcinoids were crowded in a small area of the small intestine while in others they were scattered over a long segment

Metastases were found in 25 % of the cases of gastrointestinal carcinoid The distribution of metastases is given in Table 4 Only 2 cases with multiple carcinoids had metastases

Only one case of gastrointestinal carcinoid was diagnosed on clinical grounds In that case which had widespread metastases operation had

port of such an assumption neither did the present material in which the frequency of malignant tumours was the same (about 40 % in the carcinoid material as in the entire necropsy material)

As in published series carcinoid of the small intestine was more common in men than in women in the present material

Knowledge of the incidence i e appearance of diseases in a population during a given period of time is of both theoretical and practical interest The present series of operated cases lends itself well to an elucidation of the incidence because it stems from a fairly well defined population of about 230 000 inhabitants of a town where all the inhabitants have free medical treatment at a single general hospital The town has no private hospitals The number of gastrointestinal carcinoids discovered at operation was 12 in 6 years i e somewhat less than one case per 100 000 inhabitants per year None of these operated patients had a carcinoid syndrome which agrees well with the opinion of Moertels et al that the carcinoid syndrome is rare and seen in only a very small percentage of patients with carcinoid tumour In the present biopsy series the carcinoid syndrome had been noted only once in 6 years and that was in a case of ovarian teratoma with carcinoid structures It is extremely difficult to give exact figures for the incidence of the carcinoid syndrome As a rough guess it might occur once or twice every 10 years in the population of Malmö (230 000 inhabitants)

Natural history of carcinoid All investigations on record suggest that carcinoid is a tumour which develops and grows very slowly The observations made in the present investigation argue for this assumption The operated series includes fairly many relatively young persons and in the necropsy series small carcinoids were seen in the aged It was noteworthy that in the operated series half of the carcinoids were seen in the appendix none in the necropsy series Ritchie (6) has drawn attention to this point previously and suggested that it might be due to the appendix not having been examined at necropsy This does not apply to the present material but as mentioned above all figures given here must be regarded as minimum figures There is no convincing evidence that carcinoid of the appendix occurs earlier in life than carcinoid of the small intestine It appears more likely that carcinoid of the appendix is discovered more frequently in the lower age classes owing to the wide range of indications for operation for symptoms in the region of the appendix It has also been claimed that carcinoids that grow in the narrow appendix produce clinical symptoms quicker than carcinoids of the small intestine This appears to be a plausible explanation why carcinoids of the small intestine are often not discovered until post mortem because they probably develop very slowly and asymptomatically for many years As pointed out by Moertel et al (5) on the basis of a large series of 28 cases of carcinoid followed up at the Mayo

tion in 7, while in 5 the tumour must be regarded as an incidental finding at operation

Discussion

The prevalence of gastrointestinal carcinoid in a consecutive necropsy series was found to be 53 (1 1 %) of 4828 necropsies As mentioned, the figure is difficult to compare with that found in other series Differences in examination technique, age distribution, selective factors owing to varying frequency of necropsy and the like may strongly influence the prevalence found The present material has the advantage that is was examined by uniform methods and at a hospital with a high necropsy frequency (98—99 %) This may explain why the figure found was much higher than most of those given in previous publications Thus Ritchie (6) found the frequency in a compilation of published necropsy series to vary between 0 14 % and 0 34 % Donald (2) published a necropsy series from Boston Of 18,846 consecutive necropsies at Mallory Institute gastrointestinal carcinoid had been found in 72 (0 38 %) Of 7,915 consecutive necropsies at six different Boston hospitals gastrointestinal carcinoid has been found in 0 38 % (30 cases) The similarity in frequency is remarkable In a series of 14,852 necropsies at the Mayo Clinic Moertel et al (5) found the frequency to be twice as high (0 65 %) For a Swedish series of more than 10 000 necropsies Thorson (7) reported a frequency of 0 08 % The examination of that material had most likely been very unsatisfactory and the gastrointestinal tract had rarely been slit up and rinsed His figure is surely only about one fifteenth of what it should have been The high figure found in the present material may be due in part to the high age of the necropsy series but surely mainly to the examination method As pointed out by several investigators, intestinal tumours are readily overlooked at necropsy This probably also happened in some of our cases so that the number found is probably a minimum number The highest prevalence (1 36 %) was found by Feyerter (3) in an especially meticulous search for intestinal tumours in 2500 autopsies On comparison with other series there appears to be reason to claim that the highest frequencies are the truest There is no reason to suspect over diagnosis in the present investigation because all cases were verified microscopically

The frequency (25 %) of metastasis in the present material is difficult to compare with that in other series because in most of the latter no distinction was made between biopsy and necropsy specimens The frequency of metastasis was probably low compared with that in other series in which several small carcinoids had probably been missed Ritchie (6) gives values between 21 % and 75 %

Several investigators including Moertel et al (3) supposed a relation ship between carcinoid and other malignant tumours Feyerter (4) and Thorson (7) found no evidence in sup

From Medical Department A University Hospital Copenhagen
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Organ Specific Antibodies in Addison's Disease

By J Nerup M Soborg P Halberg and K Brochner Mortensen

Tuberculous adrenalitis has become less common during the last decades whereas the relative incidence of so called idiopathic Addison's disease has increased and now it is the most common type (11 13 26)

In the discussion of the etiology and pathogenesis of idiopathic Addison's disease genetic factors have been considered but only few studies have been published (6 24)

In recent years it has been suggested *that autoimmune mechanisms might be of pathogenetic importance* for the development of idiopathic Addison's disease This assumption is supported by a number of histological serological experimental and clinical observations

(a) The adrenal cortex in idiopathic Addison's disease reveals signs of chronic adrenalitis Three histological changes are found viz round cell infiltration progressive fibrosis and epithelial changes consisting of atrophy and patchy hyperplasia (7 9 13 26) These changes are very similar to those found in chronic thyroiditis and chronic sialoadenitis in which auto immunity might be of pathogenic importance

(b) Organ specific antibody against a cytoplasmic antigen in the adrenal cortical cells have been demonstrated in sera from patients with Addison's disease (1 2 3 16 18) by an immune fluorescence technique and by a complement fixation reaction This antibody has been found with considerable frequency in sera from patients *with idiopathic Addison's disease whereas it is very unusual in sera* from patients with tuberculous Addison's disease (16)

(c) Experimental adrenalitis similar to the one found in human idiopathic Addison's disease and circulating adrenal antibodies have been produced by the injection of autologue homologue and heterologue extracts of adrenals (1 a 8 19 23 28)

(d) Several reports have been published about the coexistance of chronic thyroiditis and Addison's disease (4 5 12 22 27) Thyroid antibodies are frequently found in sera from patients with Addison's disease (3 16 18 7) *and the ultimate result of Ha*

clinic, these tumours grow and metastasize very slowly

Summary

The prevalence of carcinoids was studied in a representative 4 year necropsy series from Malmo (230,000 inhabitants) where about 60 % of all people dying in the town are necropsied in the hospital The prevalence of carcinoid of the gastrointestinal tract was about 1 1 % (53 of 4828 necropsied), the corresponding figure for bronchial carcinoid being 0 1 % Most of the carcinoids were found in the small intestine and none in the appendix In 25 % of the cases the carcinoids were multiple and in 25 % they had metastasized There were fewer cases with metastases among the multiple carcinoids (only 2 cases) All of the carcinoids except four were incidental findings

The incidence of carcinoids was studied in a 6 year series of patients operated upon at Malmo general hospital which is the only hospital in the town The incidence of carcinoid of the gastrointestinal tract was less than 1 per 100,000 inhabitants per year Most of the cases were carcinoids of the appendix The carcinoid syndrome which is very rare (and has been seen in only an extremely low per cent of all cases of carcinoid) was noted in a case of ovarian teratoma with carcinoid structures The incidence of bronchial carcinoid was somewhat less than half of that of carcinoid of the gastrointestinal tract

References

1 Collins D C Surg Gynec & Obst 101 437—445 1955
2 Donald R A Am J Med 21 867—878 1956
3 Feyerter F Erg d allg Pathologie 29 305—489 1934
4 Feyerter F Med Welt 2 912—918 1021—1027 1962
5 Moertel Ch G Sauer W G Dockerty M B & Baggenstoss A H Cancer 14 901—912 1961
6 Ritchie M B Am J Med Science 232 311—328 1956
7 Thorson A Acta Med Scand Suppl 161 1—132 1958
8 Waldenström J Verh d deutsch Ges f Verdauungs u Stoffwechselkrankheiten 20 209—219 1961

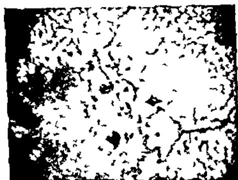


Fig 1 Unfixed section of adrenal treated by immune fluorescence technique with a serum from a patient with Addison's disease Well *defined border between marrow* without fluorescence and cortex with cytoplasmic fluorescence

guenon adrenal is of a convenient size making it possible to obtain cross sections of the whole organ in which a well defined border *between cortex and medulla is seen, thus* making the results easy to read

Microsomal thyroid antibody was demonstrated by an immune fluorescence technique as described by Holborrow et al (15) The microsomal thyroid antibody was titered by a complement fixation reaction as described by Roitt & Doniach (21) Thyroglobulin antibody was demonstrated by means of thyroglobulin sensitized sheep cells from Bourroughs Wellcome & Co

Results Tables 2—5 shows the results Sera from 35 (50 per cent) of the total number of the patients with Addison's disease contained adrenal antibody

Adrenal antibody was found in 66 per cent of sera from patients with idiopathic Addison's disease (group A) the incidence being the same in sera from male and female patients Adrenal antibody was found in sera from two of the patients with Addison's disease with known aetiology (group B) none of them had tuberculosis Two of the patients in group C had adrenal antibody in their sera One of them had had pulmonary tuberculosis 11 years before the onset of Addison's disease the other patient had a calcified hilar lymph node It is quite possible that Addison's disease *in these cases was idiopathic and not* due to tuberculous adrenalitis None of the control sera (group D) contained adrenal antibody

Thyroid antibodies were demonstrated in 42 (60 per cent) of all the patients

The most common finding was a low titre of thyroglobulin antibody The microsomal thyroid antibody was found in 27 of the patients with Addison's *disease but not in sera from the* control patients

Thyroid antibodies were found in 27 (56 per cent) of the patients with idiopathic Addison's disease (group A) All high titres of both microsomal and thyroglobulinantibodies (>16 > 2500 respectively) were found in this group Among these patients were al*most twice as many women as men* Thyroid antibodies were found in 15 (68 per cent) of the patients in group B+C but all the titres were low

No correlation was found between the occurrence of thyroid antibodies and thyroid enlargements

Discussion Although several observations *indicate that autoimmune* mechanisms may play a role in the development of Addison's disease the pathogenetic importance of the circulat

Table 1 Distribution of patients according to sex and aetiology

Sex	Total	Idiopathic Addison's disease (Group A)	Addison's disease with known aetiology (Group B)	Addison's disease and pulmonary tuberculosis (Group C)
Female	35	24	6	5
Male	35	24	9	2
Total	70	48 (68 %)	15 (22 %)	7 (10 %)

shimo's thyroiditis, viz myxoedema has been reported coexisting with Addison's disease without signs of hypopituitarism (7, 4)

Own Investigations

In the following paper a report is given of adrenal and thyroid antibody studies in 70 sera from patients with Addison's disease

Material In cooperation with a number of the medical departments in Denmark, we have so far examined 70 sera from patients with unequivocal Addison's disease

Table 1 shows the material divided according to sex and aetiology

(Group A) 48 patients were considered having idiopathic Addison's disease since no aetiology could be found Half of the patients in this group were women, half were men Eleven of the patients, 9 women and 2 men, had goitres All the patients were euthyroid

(Group B) 15 patients had Addison's disease with a known etiology 12 of these patients had tuberculous adrenalitis This diagnosis was based on autopsy findings, adrenal calcifications by x ray examination or by signs of haematogenic dissemination of tuberculosis 2 patients had tumor metastasis to the adrenal glands and in one patient the adrenals had been destroyed by panarteritis

(Group C) 7 patients had roentgenological signs of pulmonary tuberculosis In these patients tuberculosis may or may not be of aetiological importance for the development of Addison's disease Two of these patients both of them euthyroid women, had goitres

(Group D) Control sera were obtained from 29 women aged 15—70 years admitted to the hospital with gastric and duodenal ulcers, arteriosclerotic heart disease and minor mental disorders None of the patients presented signs or symptoms of thyroid or adrenal disease All of them had sedimentation rates of less than 10 mm/h

Methods Adrenal antibody have been demonstrated by an immune fluorescence technique as described by Blizzard et al (2) A typical finding in positive cases was a distinct fluorescence in all the layers of the adrenal cortex contrasting with the dark medulla (Fig 1) As previously stated by Blizzard & Kyle the antigen is organ specific but not specific of species Consequently guenon adrenal were used for the demonstration of adrenal antibodies because of the easy availability of guenons and since the

ing adrenal antibody is still unknown Only one example is known of an organ antibody with a cytotoxic effect A factor specifically toxic to cultured thyroid epithelial cells has been demonstrated in sera from patients with Hashimoto s disease and other thyroid disorders (17 20) This factor seems to be identical with the microsomal complement fixing thyroid antibody (10 14) A similar toxic effect has not been described for sera from patients with Addison s disease No reports have been published about the possible importance of delayed type hypersensitivity for the development of chronic adrenalitis

The demonstration of the adrenal antibody may be of help in ascertaining wether tuberculosis is of pathogenic importance in a case of Addison s disease because the antibody was found in 66 per cent of sera from patients with idiopathic Addison s disease but until now it has been found in no cases of unequivocal tuberculous Addison s disease in our series

The high incidence of thyroid antibodies in sera from patients with Addison s disease is in accordance with previous observations (4 7 16) These findings correspond with the frequent occurrence of thyroiditis in Addison s disease

Summary

Antibody against a cytoplasmic antigen in adrenal cortical cells was demonstrated in sera from 50 per cent of 70 patients with Addison s disease by means of an immune fluorescence technique 60 per cent of the sera contained thyroid microsomal and thyroglobulin antibody The adrenal antibody was found in 66 per cent of 48 sera from patients with idiopathic Addison s dicease but it was not found in any case of unequivocal tuberculous Addison s disease The adrenal antibody was demonstrated in no sera from individuals without Addison s disease

Acknowledgement

We wish to thank the heads of the Danish medical departments for their help and co operation in collecting the material

References

1 Anderson J R Goudie R B Gray K G & Timbury G C Autoantibodies in Addison s disease Lancet I 1123 1957
1 a Baenett E V Dumonde D C & Glynn L E Induction of autoimmunity to adrenal Gland Immunology 6 382 1963
2 Blizzard R M Chandler R W Kye M A & Hung W Adrenal antibodies in Addison s disease Lancet II 901 1962
3 Blizzard R M & Kyle M A Studies of the adrenal antigens and antibodies in Addison s disease J Clin Invest 42 1653 1963
4 Bloodworth, J M G Kirkendall W M & Carr T L Addison s disease associated with thyroid insufficiency and atrophy J Clin Endocrin. 14 540 1954
5 Brenner O Addison s disease with atrophy of the cortex of the suprarenals Quart. J Med 22 121 1928—29
6 Brochner Mortensen, K Familial occurrence of Addison s Disease., Acta Med. Scand 156 205 1956
7 Carpenter C. C. J., Solomon N Silverberg J R. Blenett, T L., Northcutt R C., Klinenberg J R Bennett I L & McGehee Harvey A Schmidts Syndrome

Table 2 Organ specific antibodies in sera from patients with idiopathic Addison's disease (Group A)

Sex	Number of Patients	Adrenal antibody	Thyroid antibodies I Microsomal antibody	II Thyroglobulin antibody	I and/or II	Adrenal and thyroid antibodies
Female	24	16	13	14	17	11
Male	24	15	7	7	10	10
Total	48	31	20	21	27	21

Table 3 Organ specific antibodies in sera from patients with Addison's disease of known aetiology (Group B)

Sex	Number of Patients	Adrenal antibody	Thyroid antibodies I Microsomal antibody	II Thyroglobulin antibody	I and/or II	Adrenal and thyroid antibodies
Female	6	1	1	2	3	0
Male	9	1	2	4	6	1
Total	15	2	3	6	9	1

Table 4 Organ-specific antibodies in sera from patients with Addison's disease and pulmonary tuberculosis (Group C)

Sex	Number of Patients	Adrenal antibody	Thyroid antibodies I Microsomal antibody	II Thyroglobulin antibody	I and/or II	Adrenal and thyroid antibodies
Female	5	2	3	4	5	2
Male	2	0	1	0	1	0
Total	7	2	4	4	6	2

Table 5 Organ specific antibodies in sera from control persons (Group D)

Sex	Number of Patients	Adrenal antibody	Thyroid antibodies I Microsomal antibody	II Thyroglobulin antibody	*I and/or II*	*Adrenal and thyroid antibodies*
Female	29	0	0	8	8	0

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On the Adrenocortical Production of Sex Hormones in Gonadectomized Rats[1]

By Stig Kullander

It has often been shown that the adrenal cortex produces steroid sex hormones and undergoes proliferative or tumourous changes in gonadectomized or old animals (for lit. se *Thung* 1962). *Frantz & Kirschbaum* (1949) who studied various strains of gonadectomized mice observed that in some of the strains the pattern of the adrenocortical hormones was dominated by oestrogen, in others by androgen. *Wolley et al.* (1941) and *Dickey & Wolley* (1949) expressed the view that the adrenocortical activity after gonadectomy starts sooner in males than in females irrespective of the age of the animals at the time of gonadectomy.

Androgen and oestrogen are possibly produced simultaneously in gonadectomized mice with adrenocortical tumour or adrenocortical hyperplasia, for female mice of the ce strain with adrenocortical tumours after gonadectomy sometimes have a submandibular salivary gland of histologically male type — a sign of androgen stimulation — in association with signs of oestrogen stimulation in other organs (*Wolley & Little* 1945).

The effect of gonadectomy on the production of sex hormones and the formation of sex hormone producing adrenal tumours has not been studied so extensively in rats as in mice. It is generally believed that adrenocortical tumours are rare in rats (*Houssay et al.* 1955, *Cohen et al.* 1957, *Iglesias et al.* 1958). *Houssay et al.* (1954, 1955) and others have, however, induced adrenocortical tumours in rats by gonadectomy. In rats of the Osborne-Mendel strain adrenocortical tumours are common, possibly because of a general endocrine imbalance in this strain, since these animals often have co-existing tumours of the adrenals, ovaries, mammary glands and pituitaries (*Snell & Stewart* 1959).

The growth of certain hormone dependent tumours such as mammary carcinoma and prostatic carcinoma in

[1] The animal operations were done in the Biolog. Dpt. Antoni v. Leeuwenhoekhuis in Amsterdam. Prof. O. Mühlbock there kindly placed laboratory facilities to my disposal.

(Thyroid and Adrenal Insufficiency) A review of the literature and a report of fifteen new cases including ten instances of coexistant diabetes mellitus Medicine *43* 2 1964
8 Colover, J & Glynn L E Experimental isoimmune adrenalitis Immunology *1* 172, 1958
9 Duffin J D Cortical necrosis of the adrenal glands associated with Addison s disease report of 8 cases Arch Path *35* 649 1943
10 Forbes I J, Roitt, I M Doniach D & Solomon I L The thyroid cytotoxic auto antibody, J Clin Invest *41* 996 1962
11 Friedmann N B The pathology of the adrenal gland in Addison s disease with special reference to adrenocortical contraction Endocrinology *42* 181 1948
12 Gastineau C F & Arnold J W Thyroid disorders in Addison s disease Proc Staff Meetings Mayo Clinic July 31 1963
13 Guttmann P H Addison s disease statistical analysis of 566 cases and study of pathology Arch Path *10* 742—85 and 895—935 1930
14 Halberg P Thyro cytotoxic factor Acta Med Scand *177* 509 1965
15 Holborow E J Brown P Roitt I M & Doniach, D Cytoplasmic localization of "complement fixing auto antigen in human thyroid epithelium Brit J Exp Path *40* 583 1959
16 Irvine W J A clinical and immunological study of adrenal insufficiency J Endocrin 26 XXXII 1963
17 Irvine W J The cytotoxic factor in thyroid disease Scot Med J 5 511 1960
18 Mead R K Autoimmune Addison s disease, New Engl J Med *266* 583 1962
19 Milcou S M Pop A Lupulescou A et Taga M L autoimmunisation experimentale de la surrenale chez le lapin. Ann d Endocr *20* 799 1959
20 Pulvertaft R J V Doniach D Roitt, I M & Hudson R V Cytotoxic effects of Hashimote serum on human thyroid cells in tissue culture Lancet *II* 214 1959
21 Roitt, I M & Doniach D Human auto immune thyroiditis Lancet *II* 1027 1958.
22 Schmidt M B Eine biglandulare erkrankung (Nebennieren und Schilddruse) bei Morbus Addisonii Verhandl Dtsch Pathol Ges *21* 212 1926
23 Steiner J W Langer B Schatz D L & Volpe R Experimental immunological adrenal injury A response to injections of autologous and homologous adrenal antigens in adjuvans J Exp Med *112* 187 1960
24 Sunder J H Bonessi J V Balash W R & Danowski T S Hereditary Addison s disease in relation to diabetes mellitus New Engl J *272* 818 1965
25 Susman W Atrophy of adrenals associated with Addison s disease J Path Bact *33* 749 1930
26 Thorn G W Dorrance S S & Emerson Day Addison s disease evaluation of synthetic desoxycorticosteroneacetat therapy in 158 patients Ann Int Med *16* 1053 1942
27 Wells H G Addison s disease with selectiv destruction of the suprarenal cortex Arch Path *10* 499 1930
28 Witebsky E & Milgrom F immunological studies on adrenal glands II Immunology 5 67 1962



Fig 1 Vaginal isograft in adrenal cortex of gonadectomized 2 month old male rat Htx eosin 5 µ. ×210



Fig 2 Isograft of ventral prostate in adrenal cortex of gonadectomized 2 month old male rat. Htx eosin 5 µ. ×700

mals killed at 2 months (Group I) this vaginal graft had formed a cyst (Fig 1) In both males and females the cysts contained some leucocytes but no cornified lamellae and the vaginal epithelium grew partly on its original stroma and partly on adrenocortical cells In both the males and the females the vaginal graft in the adrenal cortex showed roughly the same picture of inactivity and the same picture as the actual vagina *in situ* In both males and females the prostatic graft in the adrenal cortex showed roughly the same picture (Fig 2) resembling that of the actual prostatic gland *in situ* which showed signs of slight secretory activity of the epithelium with somewhat basally displased nuclei indicating mild hormonal stimulation

In the *animals that were killed at 18 months* after gonadectomy and which had had implants in the adrenals for 17 months (*Group II a*) the prostatic gland *in situ* showed only slight signs of stimulation. The vagina *in situ* showed no signs of hormonal stimulation The prostatic graft in the adrenal cortex of the males was only slightly stimulated (Fig 3) and the vaginal graft exhibited a picture of inactivity The same findings were made in the female rats On the other hand in 1 female rat in which one of the ovaries had been left *in situ* by mistake both the vaginal implant in the adrenal cortex and the vagina *showed similar hormonal stimulation* with the picture of pre oestrus

In *Group II b* in which the grafts were implanted at 17 months and *the animals killed at 18 months* the males showed signs of increased androgen activity (compared with the animals in Group II a) They showed increased stimulation of the prostatic gland *in situ* and of the prostatic implant in the adrenal cortex (Fig 4) In addition the vaginal graft in the adrenal cortex showed slight mucification Signs of slight increased androgen activity were also seen in the prostatic graft in the adrenal cortex of the fe

man can sometimes be arrested by extirpation of the adrenals in previously gonadectomized (castrated) patients. In these cases it is assumed that the operation includes *removal* of a "vicarious" source of sex hormone production (*Brown et al.* 1959). It is not known whether oestrogens are the only hormones produced or whether they simply dominate the hormone pattern in gonadectomized women and whether androgens are the only or predominant hormones produced by gonadectomized men. Nor is it known whether the interval between gonadectomy and this hormone production varies with sex.

The purpose of the present investigation was to elucidate such questions experimentally. The production of oestrogen and androgen by the adrenal cortex in gonadectomized male and female rats of different ages was studied at varying interval after gonadectomy. For this purpose small fragments of the prostatic gland and of vaginal mucosa were isotransplanted to the adrenal cortex. The histological picture of the implants was later also compared with that of the donor organs *in situ*.

Material and methods

All of the rats used were of the homozygotic R strain. Gonadectomy was done under ether anaesthesia by midline abdominal incision at 3 weeks, 17 months or 30 months. At the same time isografts of the ventral prostatic gland and vaginal mucosa respectively from 3 week old donors were implanted in the right and left adrenal cortex respectively. Some animals gonadectomized at 3 weeks did not receive the isografts until at 17 or 30 months.

Some of the animals were killed one month after implantation of the isografts while others were allowed to survive longer. The adrenals with the isografts and the prostatic glands or the vagina *in situ* were excised and fixed in 4 % formalin, embedded in paraffin and sectioned (adrenals serially) and stained with haematoxylin eosin.

In sections of the prostatic gland hormonally stimulated secretory activity was indicated by lightened endoplasmic reticulum of the epithelial cells (*Harkin* 1957). The height of the epithelium also was noted and used as a measure of secretory activity. In the vaginal sections the thickness of the epithelium and the presence of cornification or mucification were taken as indicators of hormonal influences.

All the animals were brought up in separate cages of one and the same size and on the same diet, which consisted of pellets, whole wheat once a week and tap water *ad libitum*. At death the animals were examined and careful search was made for neoplasms.

The material is summarized in Table 1.

Table 1. Experimental animals

Group	Number	Age at gonadectomy	Age at implantation of isografts	Age at death
I	5♂ + 5♀	3 w	3 w	2 m
IIa	5♂ + 5♀	3 w	3 w	18 m
IIb	5♂ + 5♀	3 w	17 m	18 m
IIc	5♂ + 5♀	17 m	17 m	18 m
IIIa	9♂ + 5♀	3 w	3 w	31 m
IIIb	5♂ + 3♀	3 w	30 m	31 m
IIIc	3♂ + 4♀	30 m	30 m	31 m
Total	37♂ + 32♀			

Results

All of the prostatic and vaginal grafts took well in the adrenal cortex. In one



Fig 7 Ventral prostate *in situ* in male rat with prostatic isograft implanted in adrenal cortex at 3 weeks in association with gonadectomy Animal killed at 31 months Htx eosin 5 μ ×700



Fig 8 Ventral prostate isograft in adrenal cortex of male rat Animal gonadectomized and graft implanted at 3 weeks Animal killed at 31 months Htx eosin 5 μ ×700

nified lammellae were observed in the vaginal graft but the vaginal epithelium was low The prostatic transplant was somewhat less stimulated than in the male animals

In *Group III a* in which *the grafts were implanted at 3 weeks in association with gonadectomy and the animals killed at 31 months* the prostatic gland *in situ* (Fig 7) showed signs of stimulation with relatively high secretorily active epithelium compared with corresponding animals but killed at 18 months At the end of this period the vagina *in situ* showed only insignificant activity of the epithelium

In male rats the prostatic graft (Fig 8) was much more stimulated than in the animals killed one year younger while the vaginal graft still showed no signs of hormonal response at all In the females of this group both the prostatic graft and the vaginal graft showed insignificant stimulation

One of the males in this group had an adrenomedullary tumour but neither the prostatic gland nor the grafts had been influenced by it



Fig 9 Ventral prostatic isograft implanted in adrenal cortex of 30 month old male rat gonadectomized at 3 weeks and killed at 31 months Htx eosin 5 μ ×700

In *Group III b* in which *the grafts were not implanted until one month before the animals were killed* the prostatic gland in situ and the vagina *in situ* showed somewhat higher hormonal activity *than in the animals in* which the grafts had been implanted at 3 weeks

In the males the prostatic graft (Fig 9) was markedly stimulated and



Fig 3 Prostatic isograft implanted in adrenal cortex of male rat at gonadectomy at 3 weeks Animal killed at 18 months Htx eosin 5 μ ×700



Fig 5 Prostatic gland of male rat gonadectomized at 17 months and killed at 18 months Htx eosin 5 μ ×700



Fig 4 Prostatic isograft implanted in 17 month old male rat gonadectomized at 3 weeks and killed at 18 months Htx eosin 5 μ ×700

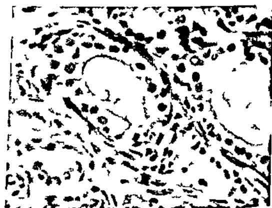


Fig 6 Prostatic isograft implanted in adrenal cortex of male rat in association with gonadectomy at 17 months Animal killed at 18 months Htx eosin 5 μ ×700

male animals In the female animals on the other hand neither the vagina *in situ* nor the vaginal graft in the adrenal cortex showed signs of stimulation

In *Group II c* in which both *gonadectomy and implantation of the isografts were performed at 17 months and the animals killed 1 months later* the prostatic gland in the males showed distended spaces with low inactive epithelium (Fig 5) The vagina *in situ* did not show such marked atrophy as in animals that had been gonadectomized for a long time — Group II a and b In the prostatic graft in the adrenal cortex in these male animals signs of stimulation were seen with a higher lighter epithelium (Fig 6) than in the actual prostatic gland In the vaginal graft however the epithelium was low In the female rats cor

gonadectomized animals has been observed by previous workers In young rats the adrenal is said to have a special andromimetic effect (*Burril & Greene* 1939) In animals that were allowed to live 18 months the androgenic as well as the oestrogenic activity was minimal (Group II a) after 31 months (Group III a) the androgen production was increased *but only in* the males

Gonadectomy at 17 or 30 months (Groups II and III c) seems to produce about the same activity in the transplants in the adrenal cortex soon after the operation as does gonadectomy before puberty (3 weeks of age)

The signs of increased stimulation observed in the prostatic grafts implanted in animals belonging to Group II and III b compared with II and III a were thus not due to increased reactivity in "younger" transplants but probably to the operations *per se* which has stimulated the steroid production by the adrenals In oophorectomized women operative stress or the injection of ACTH increased the secretion of oestrogen suggesting a latent capacity of the adrenal cortex to produce oestrogens (*Brown et al* 1959) Administration of ACTH can however also increase the formation of androgen steroids in the human adrenal cortex (*Soffer et al* 1961 *Cassner et al* (1951) found injection of large doses of ACTH in cows to increase the androgen content of the blood in the adrenal veins

The prostatic grafts sometimes showed more marked stimulation than the prostatic gland itself suggesting that the androgenic substances produced in the adrenal cortex and capable of stimulating the prostate can exert to at least a part this effect without intermediate metabolization giving a strong local effect on the transplant In the rat both transplanted prostatic fragments and vaginal fragments react to local hormonal stimuli (see *Kullander* 1956)

The slight "vicarious" oestrogen production in gonadectomized rats of this strain is remarkable as is the rarity of adrenocortical tumours It is possible that these phenomena are interdependent These R animals also have a low frequency of mammary tumours (*Boot* 1961) Of the 42 animals gonadectomized at 3 weeks and killed at 18—31 months only one was found to have a benign adrenocortical adenoma and another an adrenal medullary tumour No other tumours were found in the experimental animals

Abstract

The effect of gonadectomy on the adreno cortical production of sex hormones in male and female rats of the R strain was assessed from the growth of vaginal and prostatic grafts implanted in the right and left adrenals respectively

In both sexes gonadectomy was followed first by a slight production of androgenic substances This androgenic activity then decreased — in animals examined at 18 months it was very low — and afterwards increased — in animals studied at 31 months it was substantially higher particularly

Table 2 Degree of hormonal stimulation of prostate and vagina and of isografts of prostate and vagina implanted in adrenal cortex of the animals

Group	Sex	Prostate gland	Vagina	Graft of prostate in adrenal	Graft of vagina in adrenal
I	♂	(+)		(+)	—
I	♀		—	(+)	—
IIa	♂	[+]		[+]	—
IIa	♀		—	[+]	—
IIb	♂	(+)		(+)	(muc)
IIb	♀		—	[+]	—
IIc	♂	—		(+)	—
IIc	♀		(+)	[+]	—
IIIa	♂	+		+	—
IIIa	♀		—	—	—
IIIb	♂	++		++	(muc)
IIIb	♀		(+)	+	(muc)
IIIc	♂	—		+	—
IIIc	♀		—	(+)	—

exhibited secretory activity The vaginal graft showed mucification In the females of this group the prostatic graft and the vaginal graft was somewhat less markedly stimulated than in the males

One of the female animals in this group had an adrenocortical neoplasm a haemorrhagic cyst of the cortex with nodular cortical hyperplasia Both the vagina *in situ* and the vaginal graft in the normal adrenal cortex in that animal showed a mucosa with proliferation and mucification of the cells The prostatic graft in the adrenocortical tumour showed signs of secretion These findings are most suggestive of production of androgen by the tumour

In *Group III c* in which *the grafts were implanted in association with gonadectomy at 30 months*, and in which *the animals were killed one months later* i e at the same age as the animals in Group III a and b, the prostatic gland *in situ* showed distended prostatic follicles bordered by fairly low epithelium with flat nuclei The epithelium of the vaginal mucosa was low The picture of the epithelium of the prostatic graft in the adrenal cortex in the male rats suggested moderate activity The vaginal graft showed two layered epithelium Similar, but less pronounced pictures, were seen in gonadectomized female hosts

The results of the histological examinations altogether are given in Table 2

Discussion

The fragments of the prostatic gland and vagina implanted in the adrenals appear to be able to respond to hormonal stimulation as long as the host lives In one rat, in which by mistake only one of the gonads had been removed (Group II a) and which was killed 18 months after the implantation of the vaginal graft in the adrenal cortex the grafts showed the same cyclic phase as the vagina *in situ*

At 2 months (Group I) Rats gonadectomized at 3 weeks roughly equally in males and females showed signs of slight androgen activity of the adrenals, as judged by the histological picture of the prostatic graft in the adrenal cortex The oestrogenic activity on the other hand was extremely slight in both males and females The production of androgen in young

gonadectomized animals has been observed by previous workers. In young rats the adrenal is said to have a special andromimetic effect (*Burrill & Greene* 1939). In animals that were allowed to live 18 months the androgenic as well as the oestrogenic activity was minimal (Group II a); after *31 months (Group III a)* the androgen production was increased, but only in the males.

Gonadectomy at 17 or 30 months (Groups II and III c) seems to produce about the same activity in the transplants in the adrenal cortex soon after the operation as does gonadectomy before puberty (3 weeks of age).

The signs of increased stimulation observed in the prostatic grafts implanted in animals belonging to Group II and III b compared with II and III a were thus not due to increased reactivity in "younger" transplants but probably to the operations *per se* which has stimulated the steroid production by the adrenals. In oophorectomized women operative stress or the injection of ACTH increased the secretion of oestrogen suggesting a latent capacity of the adrenal cortex to produce oestrogens (*Brown et al* 1959). Administration of ACTH can however also increase the formation of androgen steroids in the human adrenal cortex (*Soffer et al* 1961). *Cassner et al* (1951) found injection of large doses of ACTH in cows to increase the androgen content of the blood in the adrenal veins.

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Abstract

The effect of gonadectomy on the adreno cortical production of sex hormones in male and female rats of the R strain *was assessed from the growth* of vaginal and prostatic grafts implanted in the right and left adrenals respectively.

In both sexes gonadectomy was followed *first by a slight production of* androgenic substances. This androgenic activity then decreased — in animals examined at 18 months it was very low — and afterwards increased — *in animals studied at 31 months* it was substantially higher particularly

in the males Operative procedures (stress) seemed to increase the andro genic activity of the adrenals in these animals

The production of androgenic substances did not appear to vary with the age of the animals at the time of gonadectomy (3 weeks, 17 months and 30 months)

The production of oestrogenic substances in gonadectomized rats of this strain proved low in both sexes, irrespective of age

Only few adrenal tumours were seen in the animals studied

References

Boot L M Personal communication

Brown J B Falconer C W A and Strong J A J Endocrin *19* 52 1959

Burril M W and Greene R R Proc Soc Exp Biol Med *40* 327 1939

Cohen A I Furth J and Buffert R F Am J Path *33* 631 1957

Dickey M M and Wolley G W Cancer Res 9 372 1949

Frantz M J and Kirschbaum A Canc Res *9* 257 1949

Gardner W U Cancer Res *1* 632, 1941

Gassner F, Nelson D H Reich H Rapal R T and Samuels L T Proc Soc Ex Biol Med 77, 829 1951

Harkin J C Endocrinology *60* 185 1957

Heiman J Cancer Res *4* 430 1944

Hertz R In Rec Prog Hormone Res 5 40 1950

Houssay A *Higgins* G M and Bennet W A Cancer Res *11* 297 1951

Houssay B A Cardeza A F Foglia V G Houssay A B and Pinto R M C r Soc biol *148* 918 1954

Houssay B A Houssay A B Cardeza A F *and Pinto* R M *Schweiz med Wschr* *85*, 291 1955

Iglesias R and Mardones E Brit J Cance *12* 20 1958

Kullander S Thesis Lund 1956

Snell K C and Stewart H L J Nat Cancer Inst *22* *1119* *1959*

Soffer L J Dorfman R I and Gabrilove J L The Human Adrenal Gland Philadelphia 1961

Thung P J Gerontologia *6* 41 1962

Wolley G W Fekete E and Little C C Proc Nat Acad Sci *25* *2777* *1939*

Wolley G W Fekete E and Little C C Endocrinology *28* 341 1941

Wolley G W and Little C C Cancer Res 5 203 1945

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Studies on Aldosterone Production and Sodium Metabolism in Relation to Sympathetic Nervous Function in Man

By KERSTIN HALL and BERNT HÖKFELT[1]

In the normal individual aldosterone production is closely related to sodium intake so that sodium restriction increases aldosterone production whereas sodium excess has the opposite effect (1 11) Patients with postural hypotension on the other hand have been reported to show no increase in aldosterone production following sodium restriction and to lose sodium under these conditions (1 4 18) These findings in patients with postural hypotension indicated a possible role of the autonomic nervous system in regulation of aldosterone production and sodium metabolism In order to obtain further information concerning these questions we have studied the effect of sodium restriction on urinary excretion of aldosterone and sodium in two patients with postural hypotension and in addition in two normal subjects given guanethidine (Ismelin®) and pentolinium (Ansolysen®) in doses high enough to interfere with the function of the autonomic nervous system

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Materials and methods

Case 1 (K H) a 64 year old man had a six year history of attacks of fainting in erect position, and marked fatigue and dimvision after walking only a few meters which made him resort to squatting In addition he had noted longstanding loss of sweating capacity in the left half of the body Physical examination revealed no evidence of organic heart disease and blood pressure in supine position was 185/105 mm Hg On standing for 30 seconds blood pressure decreased to 80/45 whereas pulse rate was unchanged Laboratory tests showed hemoglobin 13 0 g per cent normal serum electrolytes urea N 35 mg per cent and fasting blood sugar 87 mg per cent. The urine was free of sugar and protein specific gravity after thirst 1 023 Thyroid function was normal with PBI of 5 6 microg per cent Normal adrenal function according to clinical and laboratory findings with daily urinary excretion of 17 ketosteroids of 9—10 mg and 17 ketogenic steroids of 13—16 mg per 24 hours and with proper increase after ACTH infusion. At bed rest urinary catecholamines were normal noradrenaline ranging 7—11 microg and adrenaline 4—6 microg per 24 hours but there was no increase in urinary catecholamines in erect position. Electrocardiography at rest and x rays of skull and heart showed normal findings

Case 2 (J S) was a 63 year old man who had been operated for thyreotoxicosis in 1940 and duodenal ulcer in 1946 He was admitted

because of attacks of fainting in erect position He presented no signs of organic heart disease In supine position blood pressure was 120/80 but decreased to 55/35 after 2 minutes in erect position with practically no increase in pulse rate Hemoglobin was 12 8 g per cent serum electrolytes within normal range serum creatinine 0 9 mg per cent There was no proteinurea and maximal urinary specific gravity 1 030 Fasting blood sugar fell within normal limits but there were trace amounts of sugar in the urine in a 24 hour collection and an intravenous glucose tolerance test showed decreased glucose disappearance Thyroid function was normal with PBI 4 8 microg per cent radioiodine uptake over the thyroid 55 per cent after 24 hours and urinary excretion per 24 hours 35 per cent of administered dose Clinical status and laboratory findings showed normal adrenal function with urinary excretion of 17 ketosteroids of 15 mg and 17 ketogenic steroids 20 mg per 24 hours Urinary catecholamine excretion fell within normal limits (noradrenaline 10—12 microg and adrenaline 3—4 microg per 24 hours) No measurements were performed of catecholamine production in supine as compared to erect position Electrocardiography at rest and x rays of skull and heart showed normal findings

Case 3 (A S) was a 21 year old man admitted because of lack of puberty Final diagnosis based on surgical exploration was aplasia testis of unknown aetiology There were no signs of other endocrine dysfunctions or any other organic disease Thus physical examination of heart and circulation revealed normal findings laboratory investigations showed normal electrolytes normal PBI (5 6 microg per cent) urinary excretion of 17 ketosteroids and 17 ketogenic steroids within normal range (4—9 mg and 9—17 mg per 24 hours respectively) Urinary total gonadotrophins were high (192 ME per 24 hours) Roentgenological examinations revealed normal sella turcica but delayed closure of the epiphyseal lines (corresponding to an age of 15—16 years)

Case 4 (H A) was a 19 year old man admitted because of hypogonadism Physical examination revealed no palpable testes well developed penis scanty pubic and axillary hair and no beard Urinary total gonadotrophins were high (96 ME per 24 hours) indicating primary testicular dysfunction Laparotomy was not performed Roentgenological examinations showed normal skull and normal skeletal development for the patient's age According to clinical and laboratory investigations he had normal thyroid function (PBI 4 7 microg per cent) and normal adrenal function (urinary excretion of 17 keto steroids 10—16 mg and 17 ketogenic steroids 11—19 mg per 24 hours normal response following Metopirone® administration)

The studies were performed on the metabolic ward and the patients were allowed to be up and about during the day according to ability Throughout the studies the patients were maintained on a constant diet supplying 13 mEq sodium per day During control periods extra salt was added in an amount of 5 or 6 grams of sodium chloride per day (which corresponds to an ordinary salt intake) Following a control period sodium restriction was introduced by withdrawal of the extra salt This was done in order to test the ability of the patients to increase aldosterone production and conserve sodium To test the effect of adrenergic and ganglionic blockade on these functions Ismelin® and Ansolysen® were given to patients 3 and 4 both under conditions of extra salt and under sodium restriction Ismelin® was given orally in doses of 20—30 mg per day which dosage induced pronounced postural reactions as judged from marked fall of both systolic and diastolic blood pressure on standing concomitantly there was an increase of pulse rate Ansolysen® was given orally in increasing dosage from 10 mg to 40 mg four times a day which resulted in marked fall of systolic pressure on standing but with maintained diastolic pressure and with concomitant marked acceleration of pulse rate

For the determination of sodium potassium and aldosterone urine was collected in periods of 12 or 24 hours and stored in the cold but without preservative In cases 3 and 4 urinary analyses also included creatinine

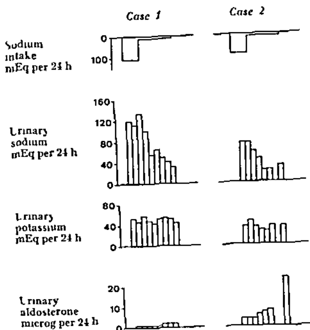


Fig 1 Effect of sodium restriction on urinary excretion of sodium potassium and aldosterone in two cases of postural hypotension

and catecholamines Sodium and potassium were determined by flame photometry using an Eppendorf spectrophotometer (2) catecholamines fluorometrically according to Euler and Lishajko ( ) For aldosterone the procedure of Neher and Wettstein (13) was used in cases 1 and 2 whereas an isotope dilution technique principally in accord with the method of Kliman and Peterson (10) was used in cases 3 and 4 For other laboratory investigations clinical routine procedures were used

Results

Effect of sodium restriction on urinary sodium and aldosterone in postural hypotension

In the two cases studied renal sodium conservation was insufficient as judged from a maximal decrease of urinary sodium to 32 and 24 mEq per 24 hours in case 1 and 2 respectively, on an intake of less than 13 mEq per day (fig 1) In both cases sodium restriction was followed by reduction of body weight probably indicating reduction of extracellular volume There were no obvious changes in serum electrolytes

Aldosterone excretion was subnormal (1—2 microg per 24 hours) in case 1 when maintained on an ordinary salt intake of 6 g per day and there was no increase in aldosterone following sodium restriction over five days Case 2 on the other hand showed urinary aldosterone (4 microg per 24 hours) within the normal range on a salt intake of 5 g per day and fol

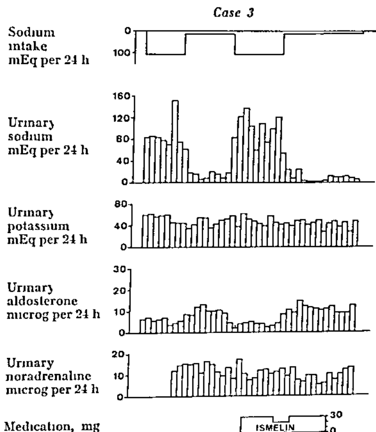


Fig 2 Effect of sodium restriction on urinary excretion of sodium potassium aldosterone and noradrenaline in a normal subject before and under treatment with guanethedine (Ismelin®)

lowing sodium restriction aldosterone increased to 24 microg per 24 hours The procedure used for the determination of aldosterone in these two patients (see above) gives urinary excretion values of 2—8 microg per 24 hours in normal subjects on an ordinary diet, with about three-fivefold increase on sodium restriction

Urinary excretion of sodium, catecholamines and aldosterone in healthy subjects during treatment with Ismelin® or Ansolysen®

On a constant sodium intake of 6 g per day urinary sodium and aldosterone remained unchanged following administration of Ismelin® and Ansolysen®, respectively (fig 2 and 3) Furthermore during medication with these drugs sodium restriction induced a marked increase in urinary aldosterone and concomitant herewith, a pronounced fall in sodium excretion which was of the same order of magnitude as seen in normal, untreated subjects There were no changes in serum sodium or potassium in connection with sodium restriction and/or treatment with Ismelin® or Ansolysen® Calculation of cumulative sodium balance during sodium restric

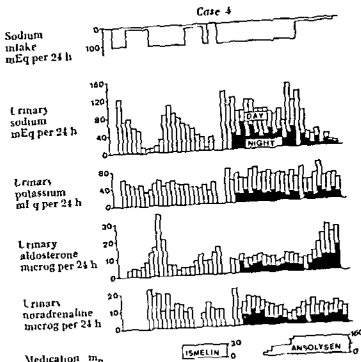


Fig 3 Effect of sodium restriction on urinary excretion of sodium potassium aldo terone and noradrenaline in a normal subject before medication and under treatment with guanethedine (Ismelin®) and pentolinium (Ansolysen®) in connection with Ansolysen® treatment urine was collected in 12 hour periods as ind cated by black areas for the night and white areas for the day

tion showed the same result indepen dent of whether Ismelin® was admi nistered or not

During treatment with Ansolysen® urinary aldosterone was measured in periods of 12 hours and it was found to be significantly higher during the day as compared to the night (fig 3)

During treatment with Ismelin® and Ansolysen® noradrenaline excre tion tended to diminish but the de crease was not statistically significant Ansolysen® did not influence the nor mally occuring diurnal variation in noradrenaline excretion which was significantly higher during the day (fig 3)

Comments

The results of the present studies in postural hypotension are *similar to* those reported by Wagner (18) and Bartter et al (1) Thus both patients failed to conserve sodium properly when put on sodium restriction Fur thermore in one of the patients uri

nary aldosterone was subnormal on ordinary sodium intake and did not increase on sodium restriction In the second patient urinary aldosterone fell within the normal range when sodium intake was 5 g a day and increased markedly during sodium restriction Sodium conservation was, however, still inefficient, which might indicate either that the increase in aldosterone production was not high enough for adequate sodium conservation or that this defect in sodium handling was not related to aldosterone production

Since postural hypotension is considered to be due to leison(s) within the sympathetic nervous system, earlier and present studies in this disease indicate that the sympathetic system can be engaged in regulation of aldosterone production and sodium metabolism The leisons can be situated at various sites such as the autonomic brain centra, the efferent spinal cord, the sympathetic ganglions or the sympathetic nerves (3, 14 15 16 17 18) which no doubt explains why the postural reaction can be connected with various signs of autonomic dysfunction A difference in location or degree of damage in the sympathetic nervous system was indicated also in our two patients and might have some bearing on the different responses in aldosterone production Thus case 1, who showed no increase in aldosterone production following sodium restriction, also presented impaired sweat regulation, whereas this was not so in case 2, who responded with increased aldosterone

Ismelin® and Ansolysen® are both potent inhibitors of sympathetic nervous function and can induce haemodynamic changes similar to those found in postural hypotension Ismelin® exerts its effect by depleting tissue catecholamines and by peripheral blockade at postganglionic nerve endings (5) It produces hypotension in normal individuals by abolishing the normal increase in peripheral vascular resistance in upright position (6) Ansolysen® on the other hand, blocks the normal ganglionic reflex In our studies treatment with Ismelin® and Ansolysen® resulted in some decrease in catecholamine excretion (although not statistically significant), and there was a marked effect on blood pressure Thus, it is evident that the doses used in our studies were sufficiently high to interfere with the sympathetic function Since treatment with Ismelin® and Ansolysen® did not interfere either with regulation of aldosterone production or sodium conservation, our studies support the view that peripheral efferent sympathetic pathways are not essential for aldosterone regulation in relation to electrolyte metabolism This is further supported by the findings that Ansolysen® medication did not alter the normally occurring diurnal variation in aldosterone excretion (12)

The lack of any overt effect on sodium metabolism of the sympatholytic drugs used in our studies were unexpected in view of the results reported by others For instance Gill et al (9) found that adrenergic blockade facilitates sodium excretion during ra

pid sodium infusion in normal subjects Furthermore Gill and Bartter (8) found that Ismelin® treatment during sodium restriction in normal individuals was followed by a cumulative loss of sodium and water

Summary

Two patients with postural hypotension showed impairment of sodium conservation ability In one of the patients aldosterone production was minimal on ordinary salt intake and did not increase on salt restriction In the other aldosterone production fell within the normal range under ordinary salt intake and increased following sodium restriction

When guanethidine and pentolinium were given to two normal subjects in an attempt to copy postural hypotension there was no evident abnormality either in sodium metabolism or aldosterone production

Acknowledgements

These investigations were supported by grants from the Swedish Medical Research Council and Stiftelsen Therese and Johan Anderssons Minne

References

1 Bartter F C Mills J H Biglieri E G & Delea C Recent Progr Hormone Res 15 311 1959
2 Bergstrom J & Hultman E Lancet 1 1132 1962
3 Birchfield R I Amer J Med 36 404 1964
4 Botticelli J T Lange R L & Kelley O A Amer J Med 37 147 1964
5 Burn, J H Brit. med J 1 1623 1961
6 Chamberlain D A & Howard Jane Brit Heart J 26 528 1964
7 von Euler U S & Lishajko F Acta physiol. scand 51 348 1961
8 Gill J R. Jr & Bartter F C J clin. Invest 43 1272 1964
9 Gill, J R Jr Mason D T & Bartter F C J clin Invest 43 177 1964
10 Kliman B & Peterson R E J biol Chem 235 1639 1960
11 Luetscher J A Jr & Axelrad B J Proc Soc. exp Biol (N Y) 8 650 1954
12 Muller A F Manning E L & Riondel A M An International Symposium of Aldosterone p 111 Little Brown Boston Mass 1958
13 Neher R. & Wettstein A J clin Invest. 35 800 1956
14 Nylin G & Levander M Ann intern Med 28 723 1948.
15 Shy G M & Drager G A Arch Neurol (Chic) 2 511 1960
16 Stead E A Jr & Ebert R V Arch intern Med 67 546 1941
17 Verel D Brit. Heart J 13 61 1951
18 Wagner H N Jr Bull Johns Hopk. Hosp 105 322 1959

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Attacks Simulating Pheochromocytoma in Patients with Angina Pectoris

By ALBERT SJOERDSMA

It is a pleasure for me to contribute in a small way to this volume honoring Jan Waldenstrom on his 60th birthday I was privileged to come to know Professor Waldenstrom via our mutual interest in the carcinoid syndrome, which he was first to recognize and relate to excess serotonin production by the tumors In 1959 I spent several months in his department and was exposed on a day to day basis to his great intellect and personality Because of his almost instinctive ability to discover clinical and pathogenetic entities, I thought it would be appropriate to discuss briefly a clinical circumstance we have observed which may be fairly common but which has not been emphasized in the medical literature This is the association of elevated blood pressure and chest pain in patients who have hypertensive episodes resembling those seen with pheochromocytoma but which may be attributed to coronary artery disease rather than excess catecholamines

On a number of occasions in the past few years I have been asked to perform chemical diagnostic tests for pheochromocytoma on urine from patients who had hypertensive attacks in association with chest pain Even though the tests were negative, the physicians in charge of these cases were often reluctant to believe that the patient did not have pheochromocytoma We have recently studied three such patients in our own institution one had actually been subjected to a negative exploratory laparotomy They were all men aged 32 59 and 62 who had repeated episodes of anterior chest pain with arm radiation accompanied by sweating tachycardia and marked blood pressure elevations Attacks lasted from a few minutes to an hour and occurred several times daily The first two patients had attacks during a prodromal period preceding myocardial infarction after which pressor episodes ceased (Fig 1) The third patient had attacks for 5 years without development of infarct Repeated observations during attacks in this patient indicated that the onset of chest pain either preceded or coincided with

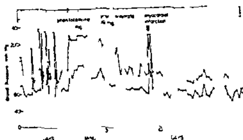


Fig 1 Hypertensive episodes occurring in association with chest pain in a 53 year old man Effects of phentolamine and glyceryl trinitrate during one episode are shown Note cessation of attacks after myocardial infarction

the onset of increases in blood pressure and heart rate

From these observations plus studies described in the literature showing the existence of pressor reflexes originating in various vascular beds we have entertained the hypothesis that the pressor attacks may be triggered by reductions in coronary flow Of course pain per se might also serve as a pressor stimulus The disappearance of pressor attacks following myocardial infarction could be interpreted as due to destruction of areas provoking the pressor reflex We also suspect that some cases of chronic hypertension may be due to coronary artery disease Certainly disappearance of chronic hypertension after myocardial infarction has been widely noted Horwitz and I have discussed this and other associations between blood pressure elevation and angina pectoris in more detail elsewhere (1)

Reference

1 Horwitz D and Sjoerdsma A Some interrelationships between elevation of blood pressure and angina pectoris *Hypertension XIII Proceedings of the Council for High Blood Pressure Research* American Heart Association Inc New York, 1965

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The fate of a Morbus Cushing Case

24 years' follow up

By HELGE B WULFF

In the history of the pathophysiology of the adrenal glands and the treatment of the adrenal diseases, the occasional isolated observation has played a dominant and stimulating rôle Ever since *Cushing's* primary findings, *Philip Hench's* (1) observation of improvement of rheumatic patients during pregnancy and in certain cases of jaundice and to *Kendall's* (2) and *Hench's* brilliant collaboration on the basis of occasional bizarre findings parallel with the *Reichstein* group all the endeavours made have lead to the final triumph — the presentation of cortisone This outstanding achievement was already begun in 1929 by *Hartmann* and *Swingle & Pfiffner*

The occasional isolated observation in the laboratory or at the bedside has by no means lost its importance The new age, that in the near future will have the electronic computer as an ensignia, is more than ever dependent on just these isolated observations for its further development However observations alone are not enough The ability to combine the observations with those things occurring in the world, often in completely different fields, is also of the greatest necessity

But also the occasional isolated recording of a course of a disease, a history over a long period of time, often makes analyses and evaluations possible which are of importance for future action

When the cortisone was introduced into clinical use in 1950 new discussions were taken up as to the possibilities of bilateral adrenalectomies in those cases of Morbus Cushing, in which the disease was supposed to be caused by hyperplasia of the adrenal cortex According to our present knowledge this is probable in approximately 60 % of these cases This method of radical therapy with the help of cortisone was soon adopted by surgeons and endocrinologists all over the world and careful recordings were made of the pre- and postoperative conditions together with the immediate risks and

possibilities The whole panorama of the disease, with special consideration to *late results of many years' duration*, however is still of importance not least in regard to possible complications

A case of Morbus Cushing followed up for more than 24 years of which more than 7 years after bilateral adrenalectomy shows a course and characteristics which might be of some *general interest*

History A previously completely healthy young nurse — *I.S born 1910* — without hereditary characteristics was in *1942* (at about 32 years of age) admitted to a University Clinic in Sweden for amenorrhea of a few months duration the cause of which could not be explained After careful investigations — including hormonal analyses — her condition was interpreted as hypophyseal insufficiency *but at the same time she showed a typical Cushing appearance* with moon face and striae and so on Blood pressure 183 120 serum calcium 10 7 mg°/o serum phosphorus 3 2 mg°/o Roentgen examination of the skeleton including the skull revealed normal sella and no osteoporosis was present Intravenous urography was normal She was given a certain stimulating and roborant treatment The amenorrhea disappeared In 1946 and 1949 she again had short periods of amenorrhea and transplantations of pituitary gland were performed Normal menstruations until the menopause in 1957

During the years 1942—1957 the patient held a demanding position as head nurse and matron at a hospital She however showed slowly increasing Cushing disease and increased 10 kg in weight Blood pressure 180/110 At the age of 45 (1955) she had two periods of mental depression During the same year she also suffered an ulceration of her lower leg with very slow wound healing which caused plastic surgery

In connection with the menopause in *1957* (47 years of age) increasing abdominal *obesity* purple complexion moon face buffalo back vascular brittleness pigmentation of the lower extremities which had become very gracile hyperglycaemia (0 20 blood sugar on fasting) total eosinopenia, increased 17 k G S A C T H test positive for Cushing increased 17 k.S Roentgen examinations of the kidneys indicated a certain hypertrophy of the right adrenal gland All findings argued for a Morbus Cushing caused by hyperplasia of the adrenal cortex

In *January 1958* (at the age of 48) right sided adrenalectomy was performed without complications (weight 8 gm microscopic diagnosis hyperplasia of the cortex)

No marked improvement was noted after the operation. In November 1958 she suffered an obscure cerebral attack with paresis of the left arm and leg which however subsided after some time Blood pressure 190/110

Considering the fact that the removal of the right adrenal did not seem to have brought about an improvement of her condition a left sided adrenalectomy was performed in *February 1959* (prep 7 gm) No complications occurred during the operation. A slight pneumothorax was corrected The Cushing picture began slowly to become normalized The blood pressure went down to 145/105 on an amount of 50 mg cortisone/day Half a year later the patient returned to work and accepted a demanding position at a hospital She was however under a heavy mental pressure with depressions and mental weakness The adequate treatment with cortisone caused marked complications and she exhibited a picture pendulating between Cushing s disease and that of Addison

First *in 1960 a definite improvement* occurred and the blood pressure became *stabilized* at 170 110 During the *years 1961—1964* she worked part time *with interruptions for mental depressions on a few occasions* In 1964 the patient suffered a slight trauma of the *right lower leg* causing a slow healing ulceration which required a long treatment During *that time also* symptoms from the abdomen with *repeated spells* of pain of unknown origin Thorough investigations gave a negative result

In May 1965 the patient was operated upon on the suspicion of acute appendicitis The appendix was healthy In the postoperative course a certain infiltration was noted in the scar

The pains continued — at times unbearable — and explorative laparotomy was performed in June 1965 A tumour like infiltrate was found in the scar (microscopic diagnosis granulation mass with necrosis of fat tissue) A band over a loop of ileum of the same appearance as the scar (microscopic diagnosis necrosis of fat tissue) was found on entering the abdomen The band was removed The patient then recovered and the abdominal pains disappeared The band and the infiltration of the abdominal wall were interpreted as having been caused by an extended cortisone treatment

The external picture of Morbus Cushing has now almost disappeared Blood pressure 130/90 Roentgen examination of the skeleton showed a moderate osteoporosis From time to time she catches colds but in general her condition is satisfactory

In a case of Morbus Cushing diagnosed in 1942 (at 32 years of age) the dominant features for 15 years were Morbus Cushing habitus, a blood pressure of 180/110 mm/Hg, a certain irregular menstrual cycle slow healing ulcerations of the lower extremities and periods of mental depression The patient, however had full working capacity for almost the whole time In 1958 a right sided adrenalectomy was performed without improvement of the patient In 1959 the left adrenal was removed Thereafter the patient experienced rather disturbing effects from the substitution therapy and for 1—2 years she was mentally very much invalidized *but there was a* clear improvement of the Cushing picture Seven years after the last adrenalectomy she showed only slight symptoms of her original disease

The substitution therapy with cortisone has for the past eight years caused the patient trouble in the form of frequent respiratory infections, periods of mental depression, slow healing wounds after slight traumata and a period of abdominal pains caused by the occurrence of a band over a loop of ileum and necrosis of the abdominal wall which may be due to the administration of cortisone over a long period of time

Due to the patient's great ambition, *she has been able to carry on her* work for almost 23 out of the 24 years that have elapsed since the onset of the disease

Conclusions

In cases of *Morbus Cushing* with certain or suspected hyperplasia of the cortex bilateral total adrenalectomy *should be performed* Unilateral procedure is advisable only in cases where a localized adenoma is demonstrable The administration of the substitution therapy requires very thorough control in regard to mental variations blood pressure nosocomial infections respiratory conditions etc A great many abnormal conditions not anticipated can develop in the digestive tract or elsewhere in the form of inflammatory *lesions necrosis and 'tumors* when administration of cortisone takes place over a *long period of time*

Complicated abnormal conditions in the region of the adrenals require con

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Complicated *abnormal conditions* in the region of the adrenals require con

tinuous control by specialists in hospitals with laboratories and other necessary equipment at their disposal. Without these possibilities surgical therapy is nowadays contraindicated.

References

1 Hench Ph S Coll Pap Mayo Clinic *8* 42 1950
2 Kendall E C Coll Pap Mayo Clinic *1* 42 1950
3 Luft R Franksson C. Nordisk larobok i kirurgi 1965
4 Moore F D Metabolic Care of the Surgical Patient W B Saunders Comp Philadelphia and London 1960
5 Scott W., Liddle H Grant W Page H A Foster J H Ann Surg *155* 696 1962
6 Skanse B Gydell K Wulff H B Koch F Acta Med Scand *119* 154 1956

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The So-Called Growth Hormone of the Anterior Pituitary

By Rolf Luft

More than 40 years ago Evans and Long demonstrated for the first time that *extracts prepared from the anterior pituitary* had growth promoting activity The substance responsible for this action was of course called growth hormone It has been purified and *shown to be a polypeptide*

By the middle of the 1950 s we had obtained a fair amount of knowledge about the widespread metabolic action of growth hormone, and it had been suggested that it *might play a role in* the homeostasis of the body s internal environment These experiments were in animals Our information about the action of growth hormone in man was surprisingly poor The reason for this was twofold Growth hormone from *animal sources had been tested in* man and though minor metabolic changes had been obtained with such preparations the results were, on the whole, disappointing Furthermore *an* accurate and easily accessible method for determination of growth hormone *in body fluids* was *not* available

These two obstacles for studies on the physiology and pathology of growth hormone *in man* were eliminated during the late 1950 s In 1956 Li and *Papkoff (7)* reported on the extraction and purification of growth hormone from human pituitaries (human growth hormone HGH), and showed that it differed in many of its *characteristics from bovine growth* hormone This preparation was highly active in man and it was established that growth hormone to some extent was species specific This was the reason for the earlier failures to obtain consistent effects with animal *growth hormones in man Further* more, the introduction by Berson and *Yalow (15) of the* radioimmunological principle for quantitative determination of insulin in blood also opened the road for quantitative determination of other hormones of polypeptide *nature in blood Today several rese* arch groups throughout the world are taking advantage of HGH determinations for clarification of the biology of the hormone

These studies have shown that in most instances the effects of HGH are *in accord with what is known from*

animal work In the following presentation these effects will be divided into two groups those related to growth and those more intimately connected with carbohydrate and lipid metabolism

Human growth hormon and growth The findings so far have clearly demonstrated the paramount effect of HGH on growth or, in a wider sense on protein synthesis (1 4) Indirect signs of anabolism have included a lowering of blood urea and a positive balance of nitrogen and potassium plus a considerable retention of sodium with expansion of the extracellular space All of these findings are consistent with the laying down of tissue Other more direct signs of the growth promoting action of HGH include the therapeutic effect of HGH when administered to children with dwarfism due to deficiency in the production of HGH by the hypophysis and the resistance to such treatment in instances where the body has produced antibodies to previously administered HGH The increased concentration of HGH in plasma from acromegalic patients and the disappearance of the hormone in blood after hypophysectomy in such patients similarly supports the growth promoting action of HGH

The mechanism of action of growth hormone in protein synthesis is only known in its rough outlines It may now be considered proven that growth hormone stimulates the initial step in protein synthesis that is the transfer of amino acids from the extracellular to intracellular compartments (5) Much less is known about the significance of growth hormone on protein synthesis after the entry of amino acids into the cells The evidence available at present — though in many respects contradictory — generally favors the concept of a direct action of growth hormone on one or several processes that regulate protein synthesis in the cell

Human growth hormone and the metabolism of carbohydrate and lipids (2 3 5 8 13) A great deal of information has been gained during the last few years regarding the action of animal growth hormone on carbohydrate and lipid metabolism On the whole these studies have added a new dimension to the earlier picture of the significance of growth hormone for metabolic processes Growth hormone administration leads to a reduction of lipid synthesis and induces a precipitous rise in plasma free fatty acid concentration due to mobilization of free fatty acid from adipose tissue (11) This important lipid mobilizing action is not a direct one on adipose tissue and the mechanisms involved remain obscure The significance of growth hormone for the further metabolism of lipids the fatty acid oxidation is not clearly understood Indirect evidence for such a stimulatory action has been suggested by the decrease in fat in the carcass of the animal given such hormone preparations the increased ketogenesis and the depression of the respiratory quotient

The important role of growth hor

mone in the regulation of *carbohydrate* metabolism has been treated extensively and comprehensively in numerous reviews in recent years (5). The anti-insulin and diabetogenic actions of the hormone were clearly demonstrated 20 to 30 years ago (16). Recently, Randle and his collegues (13) have proposed a challenging hypothesis for *the combined action of growth hormone* on lipid and carbohydrate meta*bolism. In its essence the hypothesis* favors the idea that the anti-insulin and diabetogenic effects of the hormone are secondary to its lipolytic effect. Growth hormone promotes the *mobilization of fatty acids and keto*genesis is accelerated. Since the oxidation of these substrates by muscle seems to be preferential to that of glucose, the uptake of the glucose is inhibited.

The effect of HGH on lipid and carbohydrate metabolism in man has been closely studied by our group and *several others. HGH has been shown* to exercise a 'diabetogenic' action in humans. A diminished glucose tolerance can be produced in healthy subjects by administering HGH. Fasting hyperglycemia, glucosuria, and sometimes ketonemia can be induced in hy*pophysectomized non-diabetic patients* by giving them HGH. Thus a state of 'idiohypophyseal diabetes' comparable to that found in animals is seen in *hypophysectomized juvenile diabe*tics. Very small doses of HGH produce a *dramatic deterioration of the diabe*tic state in these patients. Metabolic acidosis and insulin resistance are characteristic.

These effects of HGH are accompanied by an increased release of free fatty acids and, as shown by indirect means, by a relatively decreased peripheral uptake of glucose (3). Such a diminished peripheral *utilization of* glucose by muscle tissue following *HGH* has since been demonstrated by Rabinowitz and Zierler (12) in their studies on the human forearm.

Another aspect of the physiological role of HGH is currently being studied *and that is its significance for blood* glucose homeostasis. HGH in plasma is increased following insulin hypoglycemia (14). Plasma adrenaline, cortisol and glucagon levels are also elevated. In these tests without exception there has been a decrease in blood *sugar to values which are never en*countered in normal life and, therefore they do not necessarily give us information about the hormone's physiological significance in this respect. We may assume that the blood sugar under normal conditions, does not *fall more than 10—15 mg per 100 ml*. The response of HGH to such a minor change in blood sugar is *demonstrated* in Fig. 1 (taken from an unpublished article by Luft, Madison and *Cerasi*) (9). In this young healthy woman a blood sugar decrease of 15 mg per 100 ml (obtained by the infusion of 0.01 I.U. insulin per kg body weight for one hour) was accompanied by a remarkable HGH response, while the ex*cretion* of adrenaline was unchanged. This clearly demonstrates the pro*found effect of HGH* on blood sugar homeostasis.

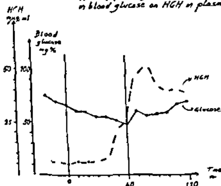


Fig. 1. Effect of prolonged small decrease in blood glucose on plasma HGH in a healthy subject

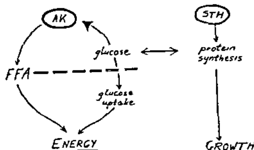


Fig. 2. The Levine-Luft two factor theory on the effect of HGH on energy metabolism and growth

The relationship between the action of growth hormone on protein synthesis and on lipid and carbohydrate metabolism. If such a relationship between the protein synthetic and adipokinetic insulin antagonistic properties of the hormone exists it has been disconcertingly obscure.

The two groups of actions may reside in one and the same molecule (10). The hormone would then be protein sparing both by its actual effect on protein synthesis from endogenous amino acids and by its anticatabolic action. Fatty acid mobilization would be a part of the latter function. Fatty acids spare glucose but this is equivalent to protein sparing during fasting since the glucose spared would otherwise have to be provided from amino acids. The theory is supported by the numerous findings that growth hormone is secreted in increased amounts when the protein stores of the body are threatened during fasting, hypoglycemia, muscular exercise, etc.

On the other hand it is rather difficult to see the biological advantage in having the adipokinetic and protein synthetic activities of the hormone reside in the same molecule especially when the production of a "growth hormone" is influenced so markedly by every day's life and in particular by even minor changes in blood sugar. For this purpose Levine and Luft (6) have introduced a theory (Fig. 2) which suggests that the so called growth hormone fraction of the anterior pituitary is composed of two hormonal entities, the *somatotrophin* proper and the diabetogenic or *adipokinetic* component. The somatotrophin would promote protein synthesis (together with insulin), cause orderly proliferation of epiphyseal cartilage and possibly lead to increased liberation of insulin from the pancreas. The total effect of somatotrophin would consist of growth in body length and enhanced protein synthesis. The adipokinetic compo

nent would stimulate the liberation of fatty acids from adipose tissue, thus shifting cellular metabolism to the utilization of fat whenever there develops a relative scarcity of carbohydrate. In this way, carbohydrate would be conserved for the use of the central nervous system which cannot readily use fat derivatives.

Future studies will show which of the one factor or two factor theories is the right one. Several reports have demonstrated the actions outlined above for the Levine Luft hypothesis. For instance, it has been shown that growth hormone preparations can and do release insulin from the β-cells in the intact dog and in man, which may explain the hypoglycemia and fall in the free fatty acid level of the blood which is seen soon after the administration of HGH.

Prolonged administration of HGH gives rise to a situation contradictory to the one just described, one in which high insulin liberation is accompanied by insulin resistance and decreased glucose tolerance. It is noteworthy that periods of rapid body growth are not accompanied by impaired carbohydrate and fat metabolism, and insulin resistance does not develop. Conversely, during fasting when carbohydrate utilization diminishes and the level of free fatty acids is increased, there is no evidence of insulin release. Such findings are difficult to explain on the basis of a one hormone theory.

This new concept for the explanation of the various actions of the so called growth hormone seems biologically advantageous. It suggests a variety of experimental approaches for testing its validity. The purification and fractioning of growth hormone preparations have to be further developed, and the hitherto sparse findings of fractions posessing one but not the other of the two activities have to be carefully tested.

Whatever such studies may show, there can be no doubt that the pituitary growth hormone has progressed from its role as stimulator of bodily growth and under special conditions, as a 'diabetogenic" agent to a key position in the regulation of body metabolism and energy homeostasis.

References

1. Beck J C McGarry E E Dyrenfurth I & Venning E H Science *125* 884 1957
2. Ikkos D & Luft R Lancet *ii* 897 1960
3. Ikkos D & Luft R Acta endocr (Kbh) *39* 567 1962
4. Ikkos D Luft R & Gemzell C A Acta endocr (Kbh) *32* 341 1959
5. Knobil E & Hotchkiss J Ann Rev Physiol *26* 47 1964
6. Levine R & Luft R Diabetes *13* 651 1964
7. Li C H & Papkoff H Science *124* 1293 1956
8. Luft R Ikkos D Gemzell C A & Olivecrona H Acta endocr (Kbh) *39* 330 1959
9. Luft R Madison L & Cerasi L (to be published)
10. Raben M S Diabetes *14* 374 1965
11. Raben M S & Hollenberg C S J clin Invest *38* 484 1959

12 Rabinowitz D & Zierler K L Nature (Lond) *199* 913 1963

13 Randle P J Garland P B Hales C N & Newsholme E A Lancet i 785 1963

14 Roth J., Glick S M Yalow R S & Berson, S A Science *140* 987 1963

15 Yalow R. S & Berson S A J clin Invest *39* 1157 1960

16 Young F G Biochem J *39* 515 1945

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1 Beck J C McGarry E E Dyrenfurth I & Venning E H Science *125* 884 1957

2 Ikkos D & Luft R Lancet *ii* 897 *1960*

3 Ikkos D & Luft R Acta endocr (Kbh) *39* 567 1962

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10 Raben M S Diabetes *14* 374 1965

11 Raben M S & Hollenberg *C S J clin* Invest *38* 484 1959

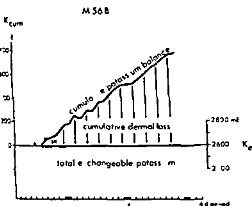


Fig 1 Cumulative potassium balance and total exchangeable potassium (x) in a 92 day metabolic study The increasing discrepancy is interpreted as caused by dermal losses of potassium

as due to dermal losses of potassium From such calculations dermal losses of potassium were estimated to 2 2—9 9 mEq per day (mean value 6 5 mEq per day) in 12 different studies

In Fig 2 another example is presented where the error introduced by dermal losses can be compared with some other systematic errors occuring in balance studies due to "invisible returns" (Isaksson & Sjogren 2) and blood withdrawn The cumulative cutaneous losses (500 mEq of potassium) were large in relation to the true change in potassium balance during the study (about 100 mEq)

Dermal losses of sodium

The dermal losses of sodium may be determined in a similar manner (Fig 3 In 7 studies performed under non sweating conditions the losses were estimated to 2 5—14 0 mEq per day Total exchangeable sodium amounts

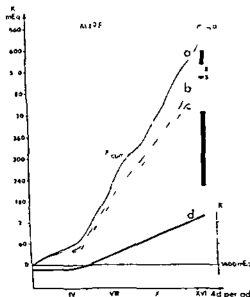


Fig 2 Systematic errors in a 64 day potassium balance study where the true changes are measured by the increase in exchangeable potassium (curve d)

Curve a = uncorrected cumulative potassium balance

„ b = cumulative potassium balance corrected for "invisible returns" (on trays pots and pans)

„ c = cumulative potassium balance corrected for "invisible returns" and blood withdrawn

The difference between curve c and curve d represents dermal losses of potassium

to only 70 per cent of total body sodium while the corresponding figure for potassium is 95 A prerequisite for our calculations is therefore that the non exchangeable fraction of bone sodium is not altered during the study

Dermal losses of calcium

The dermal losses of calcium have been estimated from the determinations of cutaneous losses of potassium

From the Metabolic Unit University of Gothenburg Sahlgrenska sjukhuset Gothenburg Sweden

Dermal Losses of Nutrients and their Significance for Human Metabolic Balance Studies

By B ISAKSSON, B LINDHOLM and B SJÖGREN

There are a great number of publications concerning the concentration of different nutrients in sweat (for references see Robinson & Robinson, 1) while reports on the daily total dermal loss of these components are considerably less frequent (Isaksson & Sjogren, 2) With the exception for sodium, these losses have been neglected in practically all human metabolic balance studies published so far The danger of misinterpretation of balance data created through this negligence was pointed out early (Mitchell & Hamilton, 3), however and recently, repeated warnings have appeared against this danger in articles by Walker (4) by Mitchell & Edman (5) and by Consolazio and co workers (6, 7 8)

At our Metabolic Unit we have worked out methods for the determination of the daily total dermal losses of potassium sodium calcium and nitrogen, which will be summarized here A few examples will show how the negligence of such losses may cause serious misinterpretation in orthodox balance studies which are based on analyses of food, urine and faeces only

Dermal losses of potassium

Recently, we accounted for a method for the indirect determination of the dermal losses of potassium (Isaksson & Sjogren, 9) The principle of the method is demonstrated in Fig 1 During an orthodox potassium balance study (Methods, see Isaksson & Sjögren, 10) total exchangeable potassium is determined repeatedly by an isotope dilution technique (Corsa *et al* 11) This gives us two different expressions for the changes in total body potassium, K_{cum} and ΔK_{ex} where K_{cum} represents the cumulative potassium balance and ΔK_{ex} the changes in total exchangeable potassium They should obviously agree but Fig 1 based on a balance study of 112 days shows that this is not the case In other long term balance studies we have regularly found similar discrepancies with respect to potassium As other systematic errors have been considered it appears reasonable to explain the discrepancy

discrepancy between the cumulative nitrogen balance and the ΔK_{ex} (Fig 4) Calculated in this way the dermal losses of nitrogen amounted to 0 2—1 6 g per day (mean 0 9 g) in 10 cases

Discussion

Our indirect determinations of the dermal losses of nitrogen have given results which are close to the figures for integumental replacement reported by Mitchell (13) In adult persons assumed to be in nitrogen balance he regularly found a positive balance of this element which was supposed to correspond to "growth of the skin and the epidermal structures" Direct measurements of nitrogen losses with sweat however give less conspicuous results presumably because desquamated epithelium is included only to a minor degree Using sweat analyses and water balance Mitchell & Hamilton (3) found figures for daily losses of nitrogen of 0 36 g per day only

Determinations of cutaneous losses of potassium and sodium from analyses of underwear plus skin washings have given results similar to our indirect estimations when sweat was collected for 24 hours (Freyberg & Grant 14 Kuutmann et al 15 Arn & Reimer 16) The figures of Dahl *et al* (17) and of Gitelman & Lutwak (18) on the other hand are considerably lower It is possible however that during these conditions electrolytes are reabsorbed from the skin and clothes Some support for this assumption can be found in Dahl's data showing the same amount of electrolytes in the clothes worn for seven consecutive days as in those worn for only three days

It is of great importance to consider dermal losses in metabolic balance studies The error introduced from negligence of these losses often accumulate to figures which are large in relation to the true changes as exemplified in Fig 1—3 The same holds true for calcium and in a few studies the apparent positive calcium balance were even changed into definite negative ones after correction for cutaneous losses of calcium (Isaksson & Sjögren to be published)

Fig 4 illustrates how a nitrogen balance study can be misinterpreted from the data of an orthodox balance It is taken from a study of the effect of anabolic steroids on the nitrogen balance in an asthmatic subject treated with cortisone The apparent nitrogen balance was positive throughout the study and accumulated to 120 g in 92 days

This should correspond to a lean tissue synthesis of 3 6 kg The observed weight increase was 3 2 kg Taking the K_{ex} figures into account however we have concluded that the increase in weight was caused by fat accumulation and/or water retention Reduction in caloric supply stopped further increase in weight but *failed* to influence the apparent positive nitrogen balance

Summary

Methods for indirect determinations of dermal losses of potassium sodium calcium and nitrogen during long

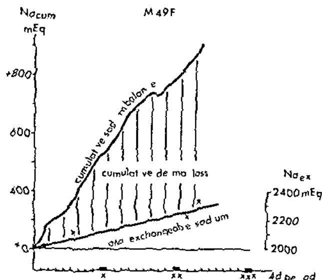


Fig 3 Cumulative sodium balance and total *exchangeable sodium (x) in a 92 day* metabolic balance study The *increasing* discrepancy is interpreted as caused by dermal losses of sodium

already described If the relation of Ca/K in whole body sweat is known the dermal losses of calcium (Ca_{derm}) can be calculated from the expression

$$Ca_{derm} = K_{derm} \times (Ca)_s / (K)_s$$

where K_{derm} stands for the daily dermal loss of potassium $(Ca)_s$ and $(K)_s$ is the concentration of calcium and potassium in sweat Data from our laboratory *have shown that in the* same person this ratio does *not* vary considerably *from day to day* nor during the day *It has* also been found *that the* ratio *Ca/K determined on* sweat samples from the arms are representative for whole body sweat (Isaksson & Sjogren to be published) The dermal losses of calcium estimated in 7 cases ranged 19—300 mg/day (mean 111 mg/day)

Dermal losses of nitrogen

Repeated *determinations of* K_{ex} *during a nitrogen* balance *study* make it *possible to estimate the* dermal losses of *nitrogen* Total *body* potassium can *be* considered as a parameter of the cell mass Changes in total exchangeable *potassium thus reflect changes* in total body nitrogen Under the assumption of a constant K/N ratio in the body (3/1 according to Elkinton & Danowski 12) the dermal losses of nitrogen can be calculated from the

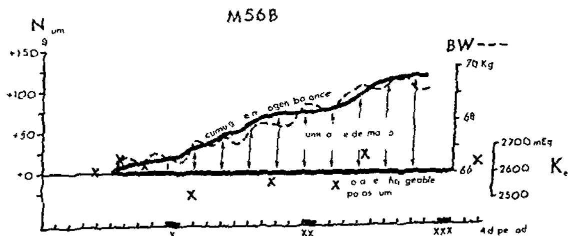


Fig 4 Cumulative nitrogen balance (———) body weight (— — —) and total exchangeable potassium (x) in the same study as in Fig 1 For explanations see text

V

MISCELLANEOUS SUBJECTS

term metabolic balance studies are described

The daily dermal losses amount to 2—10 mEq of potassium, 3—14 mEq of sodium 20—300 mg of calcium, and 0 2—1 6 g of nitrogen respectively

A few examples are given to illustrate the significance of these losses for the interpretation of balance studies

Acknowledgement

The technical assistance of Miss Britt Marie Therstol head nurse Mrs M Andersson L Bengtsson U Bengtsson technicians Miss S Lindstrand engineer and Mrs Gunilla Weimers Uddebom dietician is highly appreciated

This work was supported by the Swedish Medical Research Council (*Project No T 397 and W 410*)

References

1 *Robinson S & Robinson A H Chemical composition of sweat Physiol Rev 34* 202 1954

2 Isaksson B & Sjögren B Errors inherent in metabolic balance studies Proceedings of the 2nd international congress of endocrinology p 1041 London *1964 Excerpta Medica Foundation International* Congress series No 83

3 Mitchell H H & Hamilton T S The dermal excretion under controlled environmental conditions of nitrogen and minerals in human subjects with particular reference to calcium and iron J Biol *Chem 178 345 1949*

4 Walker A R P Uncertainties in the interpretation and validity of long term balance studies Amer J Clin Nutrition *10* 95 1962

5 Mitchell H H & Edman M Nutritional significance of the dermal losses of nutrients *in man* particularly *of nitro*gen and minerals Amer J Clin Nutrition *10* 163 1962

6 Consolazio C F Matoush R O Nelson R A Hackler L R & Preston E E Relationship between calcium in sweat calcium balance and calcium requirements J Nutrition *78 78* 1962

7 Consolazio C F Nelson R A Matoush R O Harding R S & Canham J E Nitrogen excretion in sweat and its relation to nitrogen balance requirements J Nutrition *79* 399 1963

8 *Consolazio C F* Matoush R O Nelson R A Harding R S & Canham J E Excretion of sodium potassium magnesium and iron in human sweat and the relation of each to balance and requirements J Nutrition 79 407 1963

9 Isaksson B & Sjogren B Indirect estimation of dermal losses of potassium in human metabolic balance studies Scand J Clin Lab Invest *15* supp 69 108 1963

10 Isaksson B & Sjogren B Nutr Dieta *7 175 1965*

11 Corsa I Olney J M Steenburg R W Ball M R & Moore F D The measurement of exchangeable potassium in man by isotope dilution J Clin Invest 29 1280 1950

12 *Elkinton J R & Danowski T S* The Body Fluids Williams & Wilkins Company Baltimore p 86 1955

13 Mitchell H H Adult growth in man and its nutrient requirements Arch Biochem *21* 335 1949

14 Freyberg R H & Grant R L Loss of *minerals through the* skin of normal humans when sweating is avoided J Clin Invest *16* 729 1937

15 Keutmann E H Bassett S H & Warren S L Electrolyte balance during *artifi*cial fever with special reference to loss through the skin J Clin Invest 18 239 1939

16 Arn K D & Reimer A Minimal sodium losses through the skin J Clin Invest *29* 1342 1950

17 Dahl L K Stall B G & Cotzias G C Metabolic effects of marked sodium restriction in hypertensive patients Skin electrolyte losses J Clin Invest *34* 462 1955

18 Gitelman H J & Lutwak L Dermal losses of minerals in elderly women under non sweating conditions Clinical Research *11* 42 1963

V

MISCELLANEOUS SUBJECTS

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Cerebral vascular disease

By GEORGE PICKERING
Regius Professor of Medicine University of Oxford

With the decline in the death rate from infectious diseases people are living longer and dying mainly from heart disease cancer and strokes Of these by far the least well understood is the latter

One of the main difficulties in the investigation of cerebral vascular disease is to display vessels in their continuity The standard method of slicing brains leaves large segments uninspected and it is not easy to be sure which vessels lead to which In addition a recent haemorrhage produces extensive disruption of the brain and an old lesion is difficult to identify with certainty

Three kinds of vascular disturbance may produce the sudden interruption of cerebral function which we call a stroke Embolism or thrombosis may interrupt the flow of blood haemorrhage may produce a rapidly expanding space occupying mass Embolus can usually be diagnosed only if a cause is found *e g* rheumatic heart disease with mitral stenosis or auricular fibrillation or both auricular fibrillation by itself myocardial infarction and occasionally a paradoxical embolus or a metastasis To distinguish clinically between thrombosis and haemorrhage is extremely difficult I am now convinced that I cannot do it without an angiogram

The most important advance in our understanding in this field has been the recent introduction by Ross Russell (1963) of a satisfactory method of displaying the cerebral vessels in their entirety His method had previously been developed by Mitchell and Schwartz for displaying the coronary vessels Briefly the carotid arteries were cannulated and the basilar ligated The brain was then suspended in water at 40° C for ten minutes and the arterial tree irrigated with 10% formol saline and then barium sulphate in gelatine Subsequently the hemispheres were cut coronally with a domestic meat slicer and the slices cleared by Spartheholz s method Using this method Ross Russell examined the brains of 54 subjects *freshly coming to necropsy* in Boston City Hospital the diastolic pressure was over 110 mm Hg in 16 below in 38 subjects Small aneurysms were found in all but one of the hyperten

sive group and in 10 of those with lower pressures With one exception, patients with more than 10 aneurysms all had hypertension *No aneurysms* were found in patients under 50 years These aneurysms were situated on small arteries 100—300 microns in diameter, particularly on lateral branches of the striate arteries or on penetrating vessels from the cortex The muscular tissue of the parent vessel terminated abruptly at the point of origin of the aneurysm and the remnants of elastica could be seen to extend for a short distance into the *aneurysm before disappearing* The wall of the aneurysm was composed of connective tissue only, with an inner hyaline layer derived from intima fusing with an outer collagenous layer continuous with the adventitia of the parent vessel Frequently the aneurysms showed evidence of leaking and thrombosis Ross Russel s observations suggest that such aneurysms form continuously in patients with elevated pressures as they grow older They can produce small haemorrhages and small thromboses with local softening which would resemble the miliary infarcts described by Rosenberg (1940) When the thromboses extend proximally into larger arteries larger areas of softening may result Finally there may be massive rupture with massive intracerebral haemorrhage It would seem likely that these intracerebral aneurysms are the missing link between raised arterial pressure and cerebral vascular disease

Another development of some importance has been the discovery of the role of embolism There now seems little doubt that the premonitory symptoms of carotid artery thrombosis are due to the detachment of platelet emboli from the nodule in the carotid artery (Gunning *et al*, 1964) These emboli may be so friable that they pass through the retinal and cerebral circulations, producing only a temporary interruption of the blood supply, or they may stick permanently with more lasting consequences It is quite possible that occlusions of arteries of the size of the middle cerebral, posterior cerebral or cerebellar arteries are mostly embolic from mural thrombi dislodged from nodules on the carotid *or vertebral arteries*

Thus it would seem that cerebral vascular disease is comprised of at last three separate disease processes

1 The berry aneurysms on the arteries of the Circle of Willis and the main branches arising from it These may *be congenital or mycotic* they commonly rupture to give the familiar syndrome of subarachnoid haemorrhage

2 Nodular arteriosclerosis (Councilman, 1891 Pickering 1964) of the carotid and vertebral arteries This is a *generalised* vascular disease whose most familiar manifestation is the syndrome of myocardial infarction Affecting the carotid and vertebral arteries it may produce ischaemia of the hemispheres or the brain stem possibly mostly by embolism This disease is lowly correlated with arterial pressure and is especially *frequent* in males

3 Microaneurysms of the perforating arteries of the brain These may give rise to cerebral haemorrhage or minute infarctions This disease seems to be correlated particularly with age and arterial pressure It is probably responsible for the high correlation between elevated arterial pressure and death from cerebrovascular disease

One of the tragedies of our time is a neglect of the proper use of words A technical term should describe as far as possible a fact or an idea accurately and it should have only one meaning Atherosclerosis means literally "hardening through the agency of porridge" cerebral atherosclerosis means "hardening of the brain through the agency of porridge" It would be a little difficult for a scholar unused to the misuse of technical jargon to discover that what was referred to was vascular disease of the brain But the chief crime of this term is that it confuses two diseases which are utterly different in location form and causation We shall never unravel the secrets of nature — which is supposedly the purpose of medical research — unless we learn to distinguish unlike phenomena one from another If this article has done this it will have served its purpose

References

Councilman W J (1891) *Trans Ass Amer Physcns* 6 179

Gunning A J Pickering G W Robb Smith A H T and Ross Russell R (1964) *Quart J Med* 33 155

Pickering G W (1964) *Brit med J* i 517

Rosenberg E F (1940) *Arch intern Med* 65 545

Ross Russell R (1963) *Brain* 86 425

Department of Medicine, Royal Free Hospital, London, England

Waldenstrom's Chronic Active Hepatitis

By Sheila Sherlock

In 1950, Jan Waldenstrom spoke to a meeting on Digestion and Metabolic Diseases at Bad Kissingen in the Black Forest. The title of the paper was "Leber, Blutproteine und Nahrungseiweiss". In this lecture he referred to a particularly interesting group of young people, predominantly girls during or shortly after puberty, suffering from a form of chronic liver disease. The general features were moderate jaundice with intermittent bilirubinuria and in the later stages, with ascites and oesophageal varices. In almost all cases there were spider naevi and acne. Amenorrhoea which was anovulatory, was typical. Erythrocyte sedimentation was increased. Flocculation tests were positive. Serum electrophoresis revealed that the albumin was probably normal but gamma globulin was greatly increased. The $alpha_2$ and beta globulins were maintained. This then is the first description of the condition which has since attracted much attention and been described under many names. The obscurity of the journal in which this original paper was published has given countless authors and editors difficulty in checking and the reference to it has been omitted in many publications. The paper on the same subject by Kunkel and the Rockefeller group appeared in 1951 and the other account of the same condition was by Bearn and co-workers (1956) under the title "Chronic liver disease in young women". Willocx and Isselbacher (1961) called it "Chronic liver disease in young people". A rather similar condition in women around the age of the menopause was reported by Cattan and co-workers (1957) under the title 'Cirrhoses dysprotéinémiques d'origine inconnue chez la femme'. The association with a lupus erythematosis like syndrome and with L.E. cells in the blood was noted by Joske and King (1955) and Krook (1961). Mackay and his colleagues (1956) from Australia coined the term "lupoid hepatitis". The prominent cellular infiltrate led Good (1956) to use the term "plasma cell hepatitis". Read and co-workers (1963) used the term "Active juvenile cirrhosis" although they recalled that the condition was

not always a cirrhosis in the earlier stages and did not always affect young people

Present Patients

The present material comprises 561 patients with various types of cirrhosis of the liver seen by the author at the Royal Free Hospital London between October 1959 and October, 1965 The definition of cirrhosis is widespread hepatic fibrosis with nodule formation In most instances the diagnosis has been made on histological grounds They have been divided into five groups Cirrhosis of the alcoholic (134 patients) active chronic hepatitis of Waldenstrom (139 patients) primary biliary cirrhosis (75 patients) haemochromatosis (29 patients) and Wilson s disease (13 patients) The largest group called cryptogenic (171 patients) had no significant aetiological factor Patients with biliary secondary to extrahepatic biliary tract obstruction have been excluded and also the few cases of genuine cardiac cirrhosis These 561 cases represent a spectrum of chronic liver disease seen in a hospital which has a special interest in the condition It in no way reflects the incidence of liver disease in Great Britain as a whole In fact Britain has one of the lowest incidence rates of cirrhosis in the world This paper is concerned with the active chronic hepatitis group its diagnosis course and treatment and its relation to other forms of cirrhosis seen in Britain

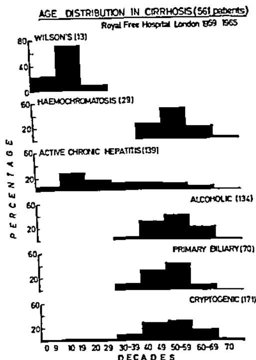


Fig 1 The age distribution of cirrhosis in the Royal Free Hospital London Note that 48 % of the 139 patients with active chronic hepatitis were between 10 and 20 years old

Criteria for Diagnosis

Clinical The condition is predominantly one of young people especially women presenting with persistent hepato cellular jaundice In the present series 48 % were between 10 and 29 years old (fig 1) This contrasts strikingly with the alcoholic cryptogenic primary biliary cirrhosis and haemochromatotic groups who have a peak incidence between 40 and 59 Nevertheless the condition can occur in childhood the youngest patient being 6 years old and also on into later life the oldest being 75 years The

Department of Medicine Royal Free Hospital London England

Waldenstrom's Chronic Active Hepatitis

By SHEILA SHERLOCK

In 1950, Jan Waldenstrom spoke to a meeting on Digestion and Metabolic Diseases at Bad Kissingen in the Black Forest The title of the paper was "Leber, Blutproteine und Nahrungs eiweiss' In this lecture he referred to a particularly interesting group of young people, predominantly girls during or shortly after puberty, suf fering from a form of chronic liver disease The general features were moderate jaundice with intermittent bilirubinuria and, in the later stages, with ascites and oesophageal varices In almost all cases there were spider naevi and acne Amenorrhoea, which was anovulatory, was typical Ery throcyte sedimentation was increased Flocculation tests were positive Serum electrophoresis revealed that the albu min was probably normal but gamma globulin was greatly increased The $alpha_2$ and beta globulins were main tained This then is the first descrip tion of the condition which has since attracted much attention and been described under many names The obscurity of the journal in which this original paper was published has given countless authors and editors difficulty in checking and the refe rence to it has been omitted in many publications The paper on the same subject by Kunkel and the Rockefeller group appeared in 1951 and the other account of the same condition was by Bearn and co workers (1956) under the title "Chronic liver disease in young women" Willocx and Issel bacher (1961) called it "Chronic liver disease in young people A rather similar condition in women around the age of the menopause was report ed by Cattan and co workers (1957) under the title "Cirrhoses dysprotéiné miques d origine inconnue chez la femme" The association with a lupus erythematosis like syndrome and with L E cells in the blood was noted by Joske and King (1955) and Krook (1961) Mackay and his colleagues (1956) from Australia coined the term "lupoid hepatitis' The prominent cel lular infiltrate led Good (1956) to use the term "plasma cell hepatitis" Read and co workers (1963) used the term "Active juvenile cirrhosis" although they recalled that the condition was



Fig 3 Active chronic hepatitis There is a profound predominantly mononuclear cellular infiltrate spreading in from the portal zones Liver cells are isolated into groups and vary in size Stained H & E×120

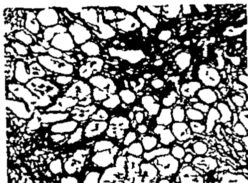


Fig 4 The liver cells are divided into groups by dense reticulin which is in parts aggregated into bands where liver cells have disappeared The zonal architecture is completely lost Stained Reticulin×120

of liver cells and eventually a post necrotic cirrhosis The fibrosis varies from portal zone enlargement through isolation of groups of liver cells in a network of dense connective tissue to frank cirrhosis with easily identifiable nodule formation The junction between liver cells and fibrous tissue is irregular the connective tissue actively encroaching on the parenchyma The early stages are those of a chronic hepatitis and later the picture is of a true postnecrotic (mainly macronodular) cirrhosis When needle biopsy material only is available because of the small sample size it may be extremely difficult to be sure whether or not a true cirrhosis is present Liver cell damage is usually profound with vacuolation of the peripheral parts of the cell cytoplasm cells varying in size and sometimes adopting a rosette like formation Giant cells may occasionally be noted Fatty change and the alcoholic hyaline of Mallory are not seen There is considerable infiltration with predominantly lymphocytes but also plasma cells both in the portal zones and also in clusters among the liver cells If autopsy material alone is studied the acute picture may have strikingly decreased and the picture may then be of a non specific post necrotic cirrhosis In most instances however search will reveal the areas of focal necrosis and cell infiltration characteristic of this form of active chronic hepatitis

Associated diseases About half the patients seem to suffer in addition from conditions apparently unrelated to the liver Arthralgia affecting the hands and larger joints appears in the particularly active case usually with fever Skin rashes of various types may be seen apart from acne These include erythemas purpuras and face lesions indistinguishable from those of systemic lupus erythematosus

The relation to ulcerative colitis is

term active juvenile cirrhosis of Read and coworkers is therefore a misnomer and the title "Cirrhosis of young people" of Willocx and Isselbacher is not always appropriate

In the present series 70 % were female (fig 2) but the occurrence in males must be emphasised and makes the title "Cirrhosis of young women" used by Bearn and co workers not always true Nevertheless, the greater incidence in females contrasts strongly with the alcoholic group In the cryptogenic group too, males are somewhat in excess (fig 2)

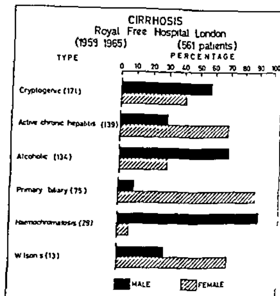


Fig 2 The sex distribution of cirrhosis in the Royal Free Hospital Note that 70 of the 139 patients with active chronic hepatitis were female

The onset is usually insidious, the patient feeling generally off colour and *is then noted to be jaundiced* In some the condition presents as a clinically quite typical attack of acute viral hepatitis and this aspect will be discussed later Icterus is a persistent feature the depth is variable the serum bilirubin level usually lying between 2 and 10 mg/100 ml This persistent jaundice of hepatocellular type *is a point of distinction from most* other varieties of cirrhosis The alcoholic is jaundiced in episodes rather than persistently and the cryptogenic type of cirrhosis is not usually associated with continued jaundice As Waldenstrom emphasised amenorrhoea and spider naevi are usual and acne is prominent in adolescence Splenomegaly is common, the liver is of variable size In spite of the jaundice the biochemical disturbance and hepatic histology, the patient looks remarkably well and is strongly built Hepatic precoma, ascites and portal hypertension are late features becoming overt only after some two to four years of illness

Biochemical The picture is of very active disease Apart from the hyperbilirubinaemia of both conjugated and unconjugated type the gamma globulins levels are very high Serum albumin is maintained until the later stages of clinical liver failure Serum transaminases are very high Towards the end transaminases and gamma globulin levels fall

Hepatic histology (figs 3, 4) The *findings on needle biopsy of the liver* are usually characteristic It is however not *infrequent to encounter a persistent* and profound increase of the prothrombin time unresponsive to intramuscular vitamin K therapy This precludes liver biopsy

The picture is of agressive fibrosis isolation and death of small groups

Treatment

Corticosteroid drugs inhibit immune and inflammatory mechanisms and may even inhibit virus replication They might therefore be expected to benefit these patients Administration does indeed give relief from fatigue decreases jaundice and reduces the serum transaminase levels The effect on serum gamma globulin values is less dramatic The effect on the underlying histological changes in the liver is difficult to determine for there is such a great difference in the appearances in different parts of the same liver Serial liver biopsies before and after corticosteroid therapy are difficult to evaluate but for what they are worth have shown that in terms of hepato cellular damage and cellular infiltration about an equal number have improved worsened or remained the same Once a true nodular cirrhosis has developed restitution of the liver to normal will be impossible whatever the treatment The evolution of portal hypertension and of liver failure are inevitable although in the individual case these may come sooner or later The life expectancy is probably little changed by corticosteroid therapy Twenty six patients with this disease died The mean duration from onset of symptoms in 12 who had received corticosteroid therapy was 3 1 years and in the 14 who had not was 1 5 years (Reid et al 1963) Infections are more frequent in the corticosteroid treated group and the facial disfiguration and bone thinning are among the other disadvantages It seemed justifiable therefore to conduct a controlled clinical trial of the value of cortico steroid therapy This is now being done at the Royal Free Hospital (Cook and Sherlock 1966) It commenced in 1963 and so far 30 patients who had never received previous corticosteroid therapy have been included in random fashion Sixteen in the control and 14 in the treated group One or two years later three of the controls are dead and three of the treated group all from liver failure or portal hypertension With regard to symptomatic benefit the cortisone treated patients seemed to be in better general condition and more able to lead a normal life This trial is clearly not complete and detailed analysis of the biochemical and histological differences between the two groups has not yet been made Further patients are being included and more time must elapse before the place of prednisone therapy can be assessed At present prednisolone should be given to those who are obviously ill deeply jaundiced and unable to lead a normal life The maintenance dose varies between 10 and 20 mg daily Every six months attempts should be made to withdraw the drug but this usually proves impossible

Other forms of immunosuppressant therapy must be considered 6 mercaptopurine (6 MP) inhibits antibody production in experimental animals and has been used to treat patients with this type of active chronic hepatitis (Page et al 1964) Four patients were treated in a dose of 1 5 mg per kg body weight and this reduced jaun

interesting, the bowel condition appearing before or after the active chronic hepatitis is diagnosed (Holdsworth et al 1956)

The kidneys are involved in a surprising proportion of cases even though routine assessment of kidney function by albuminuria, urinary deposit and creatinine *may be normal* This may be shown only by renal biopsy Twelve patients had needle biopsies of the kidney, two were normal, seven showed a glomerulitis of the type seen in systemic erythematosus, one had pyelitis, one nephrosclerosis and one hypokalemic nephropathy (Silva et al 1965)

Pulmonary infiltrates and areas of local collapse may be seen (Crispin and Lessof, 1965) Primary pulmonary hypertension has also been described (Cohen and Mendelow, 1965)

Other associations include Hashimoto's thyroiditis, diabetes mellitus rheumatic heart disease and thrombocytopaenic purpura (Read et al 1963)

Aetiological factors In about a quarter of the patients the illness seemed to begin with a typical attack of viral hepatitis In some there was even a history of preceeding contact with the disease or of occurrence in an epidemic This makes the association more possible In others however the diagnosis of viral hepatitis remains presumptive A similar histological picture in the liver can be seen among patients in a definite large epidemic of hepatitis The likelihood is that acute virus hepatitis is one initiating factor in this active chronic hepatitis of Waldenstrom

The relation to disturbed immunity has aroused increasing interest starting with the observation of Joske and King (1955) of a positive L E cell phenomenon and continuing with the observations of Mackay and co workers who used the term "lupoid hepatitis" The L E cell was in fact *seen in only about 15 % of patients* usually in those in a particularly active stage with arthralgias and fever (Read et al 1963) Apart from the activity there is no essential difference in those with a positive L E cell and those without Bouchier and co workers (1964) found other serological tests to be positive, of the antinuclear *factor in 42 % the latex fixation in* 21 % and the sheep cell agglutination in 53 %

Mackay (1961) suggested that liver cell components might become antigenic after viral or nutritional injury and so provoke an autoimmune reaction that is responsible for perpetuation of inflammation and progression *to postnecrotic cirrhosis The domi*nent lymphocytic and plasma cell infiltrate in the liver might support this theory These cells might represent "forbidden clones" which are able to damage neighbouring tissue because of their antigenicity Unfortunately destruction of *liver cells as a response* of this autoimmune process has never been confirmed and this *hypothesis* remains unproven However the wide spread involvement of various organs and the striking immunological disturbances leave no doubt that this is a systemic *disease and not one* of the liver alone

Joske R A and King W E (1955) Lancet 2 477

Krook, H (1961) Acta Medica Scand 169 713

Kunkel H G., Ahrens E H Jr Eisenmenger W J Bongiovanni A M and Slater R J (1951) J clin Invest 30 654

Mackay I R., Taft L I and Cowling D C (1956) Lancet 2 1323

Mackay I R (1961) Gastroenterology 40 617

Page A R Condie R M and Good R A (1964) Amer J Med 36 200

Read A E Sherlock S and Harrison C V (1963) Gut 4 378

Silva H., Hall E., Hill K R., Shaldon S and Sherlock S J clin Path., (1965) 18 157

Waldenstrom J (1950) Leber Blutproteine und Nahrungseiweiss Stofwechs Krh Sonderband XV Tagung Bad Kissingen p 8

Willocx R G and Isselbacher K J (1961) Amer J Med 30 185

dice and transaminase levels. These events occurred long before any decrease in the serum gamma globulin values. This suggested that the immunological reactions seen in these patients are not the cause of the liver disease but rather secondary to destruction of liver tissue by an unknown aetiological agent or a reaction to the aetiological agent itself. One patient, after a year's treatment became resistant suggesting that the drug was proving unsuitable for long term use. However, Page and co-workers remarked that this therapy does not have the drawbacks of corticosteroids. The disadvantages are of leukopenia and marrow aplasia and of infections. Further evaluation of this treatment is necessary.

Prognosis. This is extremely variable and at the outset unpredictable. *Death is most frequent during the* first four years after the recognition. Longer survivals, even up to 16 years may be seen. It may be speculated that many of the cases of cryptogenic cirrhosis may start as active chronic hepatitis. This surmise will be difficult to confirm for at autopsy, even in patients where the original hepatic biopsy showed active chronic hepatitis, the appearances are of a non specific postnecrotic (macronodular) cirrhosis.

Summary

One hundred and thirty nine patients with the Active Chronic Hepatitis of Waldenström were seen among 561 patients with cirrhosis of the liver seen at the Royal Free Hospital between 1959 and 1965. 48 % were aged between 10 and 29 years and 70 % were female. Clinically although persistently jaundiced to a varying degree, the patients remained relatively well until the terminal stages. Amenorrhoea was usual in the younger women. Biochemical changes included high gamma globulin and transaminase levels. Hepatic histology showed a very active picture with plasma and lymphocytic infiltration, aggressive fibrosis with isolation of liver cells into small groups and a postnecrotic cirrhotic.

Associated diseases were frequent and included ulcerative colitis, skin rashes, glomerulitis, pulmonary infiltrates, diabetes and Hashimoto's disease. Only some 15 % showed L. E. cells in the blood.

Aetiology is mentioned and the place of corticosteroids to relieve symptoms and jaundice is discussed.

References

Bearn A G, Kunkel H G and Slater R J (1956) Amer J Med 21 3

Bouchier I A D, Rhodes K and Sherlock S (1964) Brit Med J 1 592

Cattan R, Vesin P and Bodin H (1957) Bull et Mem Soc Hop Paris 20 608

Cohen N and Mendelow H (1965) Amer J Med *39* *127*

Cook G D and Sherlock S (1966) Unpublished observations

Crispin A R and Lessof L (1965) Brit J Radiol 38 685

Good R A (1956) Amer J Dis Child *92* 508

Holdsworth C D, Hall E W, Dawson A M and Sherlock S (1965) *Quart J* Med 34 211



Fig 1 Liver biopsy performed in October 196[illegible] Prussian blue stain for iron magnification ×60 Iron is present both in macrophages in the bands of fibrous tissue and in parenchymal cells in regenerative nodules With repeated phlebotomy over a three year period there was a complete disappearance of stainable iron in the liver

nocturia weight loss palpitations or dyspnea on exertion At the time he had glomerulonephritis in 1960 his liver was palpable 2 cm below the costal margin but tests of liver function were normal There had been no increase in skin pigmentation There was no family history of liver disease or anemia The patient was not anemic and had a normal reticulocyte count Serum iron determinations were 185 and 1[illegible]0 μg% with 100% saturation of iron binding protein Liver function was normal except for BSP retention of 17.5% (Table 1) and an electrocardiogram was normal Although fasting blood sugar levels were normal an oral glucose tolerance curve suggested latent diabetes

Since November 196[illegible] the patient has been treated by repeated phlebotomy With removal of 40 liters of blood the liver deposits of iron have been completely mobilized serum iron concentrations have fallen and the patient has become slightly anemic In October 196[illegible] liver function tests were within normal limits (Table 1) the electrocardiogram remained normal and glucose tolerance test was normal The urinary excretion of 17 ketoste

Table 1 *Laboratory findings*

	196[illegible] Oct—Dec		1963 January—December						1964 January—December							196[illegible] January—October				
	(— — — — — — — — —		— — Phlebotomy 40 liters — —																	— —)
Hematocrit	38	38	40	38	39	40	38	38	39	39	3	40	30	38	35	35	33		3	33
Serum iron	185	1[illegible]0		19												97		7		
Iron binding capacity	185	100		21[illegible]												97		307		
Bilirubin D I mg%	0 3 0 6			0 2 0 6								0 1 0								0 [illegible] 0 2
BSP retention (45 min)	17 5			2 7																1 0
Albumin (grams %)	4 0			3 7								4 1								4 1
Globulin (grams %)	3 0			2 7								3 4								3 2
Alkaline phosphatase (BU)				2 0																1 7
SGOT	39			26																

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Articular Chondrocalcinosis in a case of Hemochromatosis

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I Introduction

Articular chondrocalcinosis is a rare condition characterized by generalized calcification of cartilage This condition may involve articular or intra articular cartilage, including menisci, intervertebral discs and the pubic symphysis Articular chondrocalcinosis has been described both as an isolated entity (13), and in association with several metabolic diseases, hyperparathyroidism (3), Wilson's disease (1) ochronosis (8) or hemochromatosis Cases of hemochromatosis with chondrocalcinosis were first described by de Seze et al in 1964 (11) and four additional cases have subsequently been published by other French authors (4 6)

The case described here had arthropathy and radiographic evidence of chondrocalcinosis at least two years before hemochromatosis was diagnosed

II Observations

A 59 year old plasterer was referred to the Orthopaedic Out Patient Clinic at The Hospital for Special Surgery because of pain in the right knee The patient had been treated for hemochromatosis by repeated phlebotomy since 1962 X ray revealed generalized articular chondrocalcinosis Arthrotomy with removal of the medial meniscus was performed

A Medical History Prior to Diagnosis of Hemochromatosis

The patient had been well until 1960 when he developed *acute glomerulonephritis* diagnosed on the basis of fever hypertension edema hematuria albuminuria and renal biopsy He recovered completely from this illness without residual hypertension or evidence of chronic glomerulonephritis

B Diagnosis and Treatment of Hemochromatosis

In 1962 the patient was admitted to The New York Hospital because of a tender enlargement of the right breast Microscopic examination of excised breast tissue confirmed a diagnosis of gynecomastia An elective cholecystectomy was performed in October 1962 for asymptomatic radio-opaque gall bladder calculi At the time of operation the liver was found to be enlarged and cirrhotic and a biopsy revealed typical hemochromatosis (Fig 1) In the subsequent assessment of this problem it was found that the patient had been impotent for the previous 6 months but had suffered from no polyuria polydypsia



Fig. 4. X ray of medial semilunar cartilage removed from right knee.



Fig. 5. Section of semilunar cartilage showing birefringent deposits. Hematoxylin eosin stain viewed in polarizing microscope, magnification ×144.

siderosis. The meniscus showed degeneration of fibrocartilage and many deposits of birefringent calcific material in its substance (Fig. 5). Similar deposits were present in the articular cartilage of the femoral condyle.

4. *X ray diffraction* (E. D. Eanes, Ph.D.) of a fragment of the bony mass removed from the antero medial compartment revealed the presence of randomly oriented micro crystals of a mineral apatite and failed to show evidence of any distinct crystalline pyrophosphate.

5. *Blood chemistry.* Latex fixation tests in 1960, 1963 and 1965 were negative; serum calcium and phosphate and alkaline phosphatase analyses in 1960, 1963 and 1965 were normal. Serum uric acid analyses performed in 1960 were reported as 7.5, 4.8, 7.1, 4.8 and 5.0 mg per 100 cc, and in 1965 4.4 mg per 100 cc. Only two values were thus higher than the normal of 6.0 mg per 100 cc.

III. *Discussion*

It is well known that synovial deposition of hemosiderin occurs in hemochromatosis but this manifestation of the disease has never been shown to produce clinical signs of arthropathy. However, recently de Sèze et al. (11) published 6 cases, Delbarre (4) 2 cases and Jaffres and Kerbrat (6) 2 cases of hemochromatosis with arthralgia and articular chondrocalcinosis diagnosed radiographically. The case published here is particularly interesting because chondrocalcinosis was shown to be present prior to the onset of symptoms attributable to hemochromatosis and because analyses of cartilage, bone and one meniscus showed the presence of calcium deposits without iron. However, the synovium contained iron whereas the liver no longer did. This was found also in the case reported by Kra et al. (7).

Although the association of articular chondrocalcinosis with hemochromatosis has only recently been recognized, this is probably not rare. By systematic radiographic examination of the joints in 6 cases of hemochromatosis de Sèze et al. (11) found 2 cases of chondrocalcinosis and Schumacher (9) found 5 cases of arthralgia in a review of 23 cases of hemochromatosis. Arthralgia in patients with hemochromatosis may be neglected be



Fig 2 X ray of right hip in 1965 Calcification of articular cartilage produces a line concentric to the bone

roids was 7.0 mgm in 24 hours (normal for male 15 mgm per 24 hour urine)

C *Arthralgia and Diagnosis of Chondrocalcinosis*

The patient has suffered from intermittent pain in several joints including the right shoulder and both knees since 1958 In 1960 *when he was admitted with glomerulonephritis* he complained of pain in the knees and both joints were tender warm and swollen

In 1965 the patient was referred for orthopaedic care because of pain in the right knee of several months duration *there* was no history of trauma He walked with a slight limp and he could not perform a deep knee *bend* The right knee had a loss of 10° full extension slight effusion a palpable mass in the medial compartment *anteriorly slight ten*



Fig 3 X ray of right knee in 1965 Both semilunar cartilages are visible because of calcification

derness on palpation of the medial joint line but was otherwise normal on physical examination as were *all other joints*

1 *X ray examination of the joints* in 1965 revealed generalized cartilage radiodensity especially apparent in shoulder hip knee joints and in the semilunar cartilages in the *intervertebral discs of the lumbar spine in* the pubic symphysis and in the triangular cartilage of the radiocarpal joints but not present in the finger joints Review of x rays of 1960 revealed that this condition was present *even at that time i e two years prior to* the diagnosis of hemochromatosis (Figs 2 3)

2 *Arthrotomy of the right knee joint with removal of the medial meniscus* was performed because of the extension defect and tenderness at the medial joint line The *joint* contained some 50 cc of viscous dark yellow clear fluid The synovium was not thickened and had normal color The cartilage at the femoral and tibial condyles was encrusted with a white material and in a *few spots* white hard material was deposited on the cartilage surface Both menisci were likewise encrusted (Fig 4) the medial meniscus was frayed at its central border A bony hard piece of *tissue 1 × 1* cm large *was attached to* the anterior horn of the medial meniscus

3 *Histologic examination* (Robert C Mellors M D Ph D) of the synovium showed deposits of hemosiderin in synoviocytes and *macrophages diagnostic of synovial hemo*

other radiographic signs of chondrocalcinosis hemochromatosis should be considered as a possible diagnosis

V Acknowledgement

Supported by U S Public Health Service Training Grant AM 5414 and U S P H S Grant 5K3AM 14 153

References

1 Boudin G Pepin B et Hubault A Les arthropathies de la maladie de Wilson Rev Rhum nr 10 594—598 1964

2 Bundens W D Jr Brighton C T and Weitzman G Primary articular cartilage calcification with arthritis (pseudogout syndrome) J Bone and Joint Surg 47 A 111—122 1965

3 Bywaters E G L Dixon A St and Scott J T Joint lesions of hyperparathyroidism Ann rheum Dis 22 171—187 1963

4 Delbarre F Les manifestations o téo articulaires de l'hémochromatose Presse méd 72 50 2973—2978 1964

5 Hosking G E and Clennar C Calcification in articular cartilage J Bone and Joint Surg 42 B 530—534 1960

6 Jaffres R et Ketbrat G Calcinose articulaire diffuse chez deux frères atteints d'hémochromatose Rev Rhum 32 431—438 1965

7 Kra S J Hollingsworth J W and Finch S C Arthritis with synovial iron deposition in a patient with hemochromatosis New Engl J Med 272 1268—1271 1965

8 McCollum D E and Odom G L Alkaptonuria ochronosis and low back pain A case report J Bone and Joint Surg 47 A 1389—1392 1965

9 Schumacher H R Jr Hemochromatosis and arthritis Arthritis and Rheumatism 7 41—50 1964

10 Selye H "Calciphylaxis" Univ of Chicago Press 1962

11 Sèze S de Hubault A, Welfling J Kahn M F Solnica J Les arthropathies des hémochromatoses Rev Rhum 31 479—485 1964

12 Wolke K Über meniskus und gelenkknorpelverkalkungen Acta radiol (Stockh) 16 577 1935

13 Zitňan D and Sitaj S Chondrocalcinosis articularis Ann Rheum Dis 22 142—170 1963

cause of the more severe manifestations of this disease. Advanced chondrocalcinosis, however, may cause no symptoms; our patient has no symptoms of hip disease in spite of his radiographic signs.

From the published case reports the menisci appear to be the most common site of chondrocalcinosis in hemochromatosis (4, 6, 11), as in idiopathic chondrocalcinosis (13). In one of Schumacher's (9) two cases and in a case published by Kra et al. (7) meniscal calcifications were noted even though chondrocalcinosis otherwise was not discussed in these two publications. Calcified menisci are rarely found in any of the common arthritides. Delbarre (4) and Hoskin and Glennair (5) quote three authors who found meniscal calcifications in about 0.3% of radiograms of the knee. Five of 8 cases reported by one author (12) had radiographic evidence of chondrocalcinosis of other joints. X-ray evidence of meniscal calcifications is thus highly suggestive of chondrocalcinosis and whenever this is seen the associated metabolic diseases should be considered in the differential diagnosis.

The etiology of chondrocalcinosis in hemochromatosis is not known. The absence of iron deposits in the cartilage in our patient and the presence of trace amounts only in a patient described by de Seze et al. (11) suggest that the local accumulation of iron is not important. By way of speculation attention should perhaps be drawn to Selye's (10) experimental studies of calcifylaxis; he was able to precipitate tissue calcium deposits by local injections of iron and other metal salts. However, this was possible only after administration of toxic doses of vitamin D.

Bundens et al. (2) have recently stressed that in cases of idiopathic articular chondrocalcinosis with arthritis ("Pseudogout Syndrome") the joint fluid contains calcium pyrophosphate identifiable by x-ray diffraction. In the case described here the joint fluid did not contain any crystals, and x-ray diffraction of an intra-articular calcific deposit suggested the presence of calcium apatite rather than calcium pyrophosphate.

IV. *Summary*

A 59-year-old man had arthropathy and radiographic evidence of generalized chondrocalcinosis two years before hemochromatosis was diagnosed. During a three-year period repeated phlebotomy has completely removed iron from the liver and the patient has developed no further complications of hemochromatosis.

At this time knee arthrotomy with meniscectomy was performed. The articular cartilage and meniscus contained calcium but not iron. X-ray diffraction of calcific deposits suggested the presence of apatite rather than pyrophosphate structure.

Comparison with previously published cases of chondrocalcinosis associated with hemochromatosis indicates that this association may not be rare though described only recently. In patients with meniscal calcifications or

Table I. *Age and sex distribution of present SLE series and of two American SLE series*

Series	Age at diagnosis, years								Total	Sex	
	5–9	10–19	20–29	30–39	40–49	50–59	60–69	70–79		Males	Females
Present series	0	1	10	16	13	5	7	2	54	6	48
%	0.0	1.8	18.5	29.6	24.1	9.3	13.0	3.7	100.0	11.1	88.9
S. of Merrell and Shulman	0	10	20	29	27	7	6	0	99	13	86
%	0.0	10.1	20.2	29.3	27.3	7.1	6.0	0.0	100.0	13.1	86.9
S. of Kellum and Haserick	3	47	81	70	55	22	18	3	299	42	257
%	1.0	15.7	27.1	23.4	18.4	7.4	6.0	1.0	100.0	14.0	86.0

Table II. *Data for calculation of survivorship, present SLE series*

Yr after diagnosis	Nr observed x or more Yr after diagnosis	Nr last observed and alive in interval between this x and next stated x Yr	Nr dying in interval between this x and next stated x Yr	Estimated proportion of persons surviving to this x who		Percentage of persons diagnosed who survive x Yr
				die before next stated x Yr	survive to next stated x Yr	
(x)	(O_x)	($_nw_x$)	($_nd_x$)	($_np_x$)	($_nq_x$)	(P_x)
(1)	(2)	(3)	(4)	(5)	(6)	(7)
0	54	0	10	0.185	0.815	100.0
1	44	0	2	0.045	0.955	81.5
2	42	0	3	0.071	0.929	77.8
3	39	1	0	0.000	1.000	72.3
4	38	5	1	0.028	0.972	72.3
5	32	2	2	0.065	0.935	70.3
6	28	4	1	0.038	0.962	65.7
7	23	4	1	0.048	0.952	63.2
8	18	4	1	0.063	0.937	60.2
9	13	4	1	0.091	0.909	56.4
10	8	3	1	0.154	0.846	51.3

tively) whereas the present material was composed only of Scandinavians. All patients except three had been treated with corticosteroids (94.6 %) compared with 75.8 % of the patients of Merrell and Shulman and 90.0 % of the patients of Kellum and Haserick.

The data for calculation of survival are given in Table II. Column 5 represents the estimated mortality for each year after diagnosis and column 7 the cumulative survival percentages. It is seen that *70.3 %* of the patients were expected to survive for five years and

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Long-Term Prognosis of Systemic Lupus Erythematosus

By Tore Leonhardt

Statistical evaluations of the long term prognosis of systemic lupus erythematosus (SLE) have been published by Merrell and Shulman (1955) and by Kellum and Haserick (1964) Since no such study seems to have been made of European materials of SLE, it was considered worth while to present the results of a follow up of 54 Swedish patients with SLE

Material and methods

The material consists of a series of patients with SLE seen at hospitals in Scania 1955—1961 It was originally collected to study genetic factors in the etiology of SLE (Leonhardt 1964) Perusal of registers of hospital inpatients biopsy and necropsy files of departments of pathology and LE cell registers of hospital laboratories revealed 113 cases with a clinical picture consistent with SLE Of these 59 were considered to have "definite" SLE having at least one diagnostic criterion in the form of "pathognomonic" necropsy findings characteristic skin changes *or* a positive LE cell phenomenon *and* a medical history judged as typical of SLE The remaining 54 cases had a less firm diagnosis and were not included in the present study since they were not thought to be comparable with the SLE patients selected by the above mentioned authors

In five of the 59 cases *with definite* SLE the diagnosis was not established until post mortem These cases were not relevant to a study of long term prognosis and were therefore excluded In the remaining 54 cases the duration of the survival was calculated from the date of *diagnosis to death or date of last* observation of those still living The diagnosis was taken as the starting point because it is difficult to date the onset of SLE in a given case (Merrell and Shulman 1955 Leonhardt 1964) The follow up was discontinued in November 1965

The statistical method described in detail by Merrell and Shulman (1955) and also used by Kellum and Haserick was chosen for the present study The probability that a person alive in a stated year after diagnosis will die before the next year was estimated by application of the following formula to the data (symbols see Table II)

$$_nq_x = \frac{_nd_x}{O_x - \frac{1}{2}\,_nw_x}$$

Results

Of the 54 SLE patients six (11 1 %) were males the material thus showing the usual preponderance of SLE in females (Table I) The age distribution is also shown *in Table I In the series* of Merrell and Shulman as well as in the series of Kellum and Haserick the patients were on the whole younger Both American series included Negroes (26 3 % and 7 7 % of total respec

Table I *Age and sex distribution of present SLE series and of two American SLE series*

Series	Age at diagnosis years								Total	Sex	
	5–9	10–19	20–29	30–39	40–49	50–59	60–69	70–79		Males	Females
Present series	0	1	10	16	13	5	7	2	54	6	48
%	0 0	1 8	18 5	29 6	24 1	9.3	13 0	3 7	100 0	11 1	88 9
S of Merrell and Shulman	0	10	20	29	27	7	6	0	99	13	86
%	0 0	10 1	20 2	29.3	27.3	7 1	6 0	0 0	100 0	13 1	86 9
S of Kellum and Haserick	3	47	81	0	55	22	18	3	299	42	257
%	1 0	15 7	27 1	23 4	18 4	7 4	6 0	1 0	100 0	14 0	86 0

Table II *Data for calculation of survivorship present SLE series*

Yr after diagnosis	Nr observed x or more Yr after diagnosis	Nr last observed and alive in interval between this x and next stated x Yr	Nr dying in interval between this x and next stated x Yr	Estimated proportion of persons surviving to this x who die before next stated x Yr	Estimated proportion of persons surviving to this x who survive to next stated x Yr	Percentage of persons diagnosed who survive x Yr
(x)	(O_x)	($_nw_x$)	($_nd_x$)	($_np_x$)	($_nq_x$)	(P_x)
(1)	(2)	(3)	(4)	(5)	(6)	(7)
0	54	0	10	0 185	0 815	100 0
1	44	0	2	0 045	0 955	81 5
2	42	0	3	0 071	0 929	77 8
3	39	1	0	0 000	1 000	72.3
4	38	5	1	0 028	0 972	72.3
5	32	2	2	0 065	0 935	70.3
6	28	4	1	0 038	0 962	65 7
7	23	4	1	0 048	0 952	63.2
8	18	4	1	0 063	0 937	60 2
9	13	4	1	0 091	0 909	56 4
10	8	3	1	0 154	0 846	51.3

tively) whereas the present material was composed only of Scandinavians All patients except three had been treated with corticosteroids (94 6 %) compared with 75 8 % of the patients of Merrell and Shulman and 90 0 % of the patients of Kellum and Haserick

The data for calculation of survival are given in Table II Column 5 represents the estimated mortality for each year after diagnosis and column 7 the cumulative survival percentages It is seen that 70.3 % of the patients were expected to survive for five years and

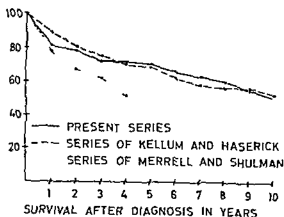


Figure 1 Survival of patients with systemic lupus erythematosus

51 3 % for ten years after diagnosis Similar figures were found by Kellum and Haserick (69 4 % and 53 7 % respectively), while Merrell and Shulman observed a higher mortality only 51 5 % of their patients were estimated to survive for four years after diagnosis (the patients not being followed long enough to calculate the five or the ten year survival rate) The estimated survival rates calculated for the three materials in comparison are given in a graphic form in Figure 1

The high mortality during the first year after diagnosis in all three materials is due to the fact that many patients were not hospitalised and diagnosed until the acute onset of a severe SLE crisis In the subsequent years the annual mortality was significantly lower and relatively constant

In most diseases, survival is influenced by the composition of the material as to sex age and other variables According to Kellum and Haserick the prognosis of SLE is poorer among males than among females The course of SLE is held to be particularly unfavourable in children (Cook et al 1960) on the other hand, aged patients run an increased risk of being affected by other diseases which lower vitality Racial differences in course and outcome of SLE may exist, but hitherto published data (Kellum and Haserick 1964) are inconclusive Differences with regard to such variables make strict comparisons between the three mentioned SLE series impossible It is however interesting to note the close survival curves and the almost identical figures for five and ten years survivorship in the present series and in the series of Kellum and Haserick

The data of Kellum and Haserick indicated that the prognosis of SLE may be improved by corticosteroid treatment, although the small number of untreated patients did only permit any valid conclusions for the first two years after diagnosis Thus the higher mortality found in the series of Merrell and Shulman than in the present series and in that of Kellum and Haserick might, at least partly, be explained by the fact that one fourth of their patients did not receive hormone treatment against only five and ten percent respectively, of the patients in the other two series

The survivorship figures must be interpreted in the light of the general death rate in the population from which the patients derived According to the 1958 life tables in Sweden (Statistisk Årsbok för Sverige 1964), the survival rate of a group of the Swedish population matched to the present

SLE series as to sex and age would be 97.3 % after five and 93.6 % after ten years.

Although the introduction of cortico-steroid treatment has made it possible to influence the course of SLE favourably, SLE must still be regarded as a serious disease with a dubious long-term prognosis. Only about half of the patients in a series of definite SLE cases can be expected to survive for ten years after diagnosis.

Summary

The long-term prognosis of systemic lupus erythematosus (SLE) was studied in 54 Swedish patients. The estimated survival after diagnosis was 70.3 % after five years and 51.3 % after ten years. The life expectancy of a sex and age matched Swedish population was 97.3 % and 93.6 % respectively. The results were compared with the survival rates in two American SLE series.

References

Cook, C. D., Wedgwood, R. J. P., Craig, J. M., Hartmann, J. R. & C. A. Janeway: Pediatrics *26*, 570, 1960.

Kellum, R. E. & J. R. Haserick: Arch. Int. Med. *113*, 200, 1964.

Leonhardt, T.: Acta med. Scand. Suppl. 416, 1964.

Merrell, M. & L. E. Shulman: J. chron. Dis. *1*, 12, 1955.

Statistisk årsbok för Sverige. Norstedt & Söner, Stockholm 1964.

The investigation was supported by a grant from *Anders och Carl Erikssons fond*.

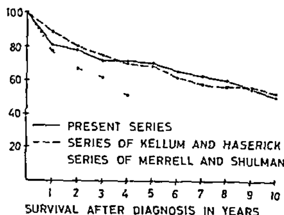


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Fig 1 Per bronch al vessels packed with eos noph l cells



Fig 3 Eosinoph l c granuloma (arrow) in the per bronchial t ssue



Fig 2 Tran gress on of the essel wall by eo nopl ls wh ch are scatterad n the mucosa

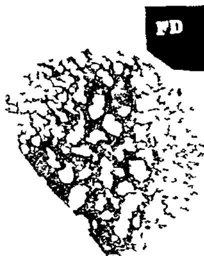


Fig 4 Marg nal emphysema and eos no phil c inf ltrat on of the lung

From the "Margareta and Erik Wahlborg Laboratori" for Research in Allergy Helsingborg (Sweden)

Polyangitis in Allergic Conditions

By P Kallos and L Kallos-Deffner

In a *stimulating* paper, presented at the Second Symposium of the Collegium Internationale Allergologicum in Basle in 1956, *J* Waldenström stated that more or less widespread periarteritis nodosa ("polyangitis") has, at least in a certain number of cases, an allergic etiology Furthermore, he described enlightening case histories, showing that spontaneous *remissions of polyangitis* are "by no means rare Intensive treatment with corticosteroids can have either a favourable temporary effect, or cure even long lasting and severe cases of this condition

At autopsy of asthmatic patients who died in status asthmaticus, the occurrence of periarteritis nodosa was first noted by E Bahrmann in 1935 Later many such cases were described and we refer to the review by E Churg and L Strauss Recently K F W *Hinson* claimed that arteritis (excluding areas of infection and infarction), i e necrosis of the vascular wall and a *local inflammatory* response" with intense eosinophilia can be found at autopsy in asthmatics He is inclined to suppose that Löffler's eosinophilic infiltration (cf 10, 11) allergic granulomatosis, Wegener's syndrome and polyarteritis nodosa are 'imperceptibly merging conditions of increasing severity"

The examples given in Waldenstrom's *paper allow the assumption* that febrile episodes with blood eosinophilia in the course of asthma drug allergy *and other allergic conditions* are the first symptoms of polyangitis These can have a grave prognostic significance *(15, 3)*

In experimental allergic asthma in guinea pigs induced by inhalation of the aerosolized specific antigen we could show (5 6 7 8) that already after one severe asthmatic attack of short duration (3—10 minutes) the bronchial vessels are packed with eosinophil cells (fig 1) After some consecutive attacks (3—5 inhalations) the eosinophils infiltrate and transgress the vascular wall and invade the surrounding tissue (fig 2) The eosinophils form granulomas (fig 3) or after several asthmatic attacks (up to ten) marginal infiltrations in the lungs (fig 4)

8 Kallós P and Pagel W Experimentelle Untersuchungen über Asthma bronchiale Acta med scand *91* 292 (1937)

9 Leopold J G A contrast of bronchitis and asthma In "The nature of asthma" report of a Symposium held at the King Edward VII Hospital Midhurst (England) May 1964

10 Löffler W Transient lung infiltrations with blood eosinophilia Int Arch Allergy *7* 300 (1955)

11 Meyenburg H v Ueber eosinophile Lungenentzündungen Schweiz Z Tbk *1* 205 (1944—45)

12 Noelpp Eschenhagen I and Noelpp B New contributions to experimental asthma Progr Allergy *4* 361 (1954)

13 Pagel W Pathologie und Histologie der allergischen Erscheinungen Progr Allergy *1* 73 (1939)

14 Pagel W and Treip C S Viscero cutaneous collagenosis J clin Path *8* 1 (1955)

15 Waldenström J Some clinical aspects of the diagnosis and treatment of vascular allergy Int Arch Allergy *7* 309 (1955)

The lungs show, at the same time, emphysematous and atelectatic changes, resembling those recently described by J G Leopold in humans These are certainly allergic changes Already in 1937 we could show (8) that the inhalation of histamine- or acetylcholine aerosol in guinea pigs induces the clinical picture of asthma but never the above described histological changes (cf also 12) As we (5, 6, 7, 8) and W Pagel (13) pointed out, in these asthmatic guinea pigs not only the lungs but other organs such as the heart and spleen show similar changes which must be considered as the first stages of allergic polyangitis In spite of their severity and extent, these experimentally induced changes in asthmatic guinea pigs are completely reversible Spontaneous recovery occurs in about 30—50 p C of guinea pigs exposed at least ten times "Tuberculoid" granulomas, infarction and scars occur then in the affected tissues

Stimulated by the clinical observations of J Waldenström we could show (4 and unpublished observations) that by carefully planned treatment with corticosteroids the rate and speed of recovery of asthmatic guinea pigs can be enhanced considerably We also pointed out as J Waldenström did the close connection of the described changes with alterations of the connective tissue This constitutes a link between this group of allergic conditions and the s c connective tissue- or collagen diseases In the latter polyangitis is a common feature (14) In circumscribed and extremely intense biphasic allergic lesions ("anaphylactic myositis") and as a sequele of challenging allergic animals simultaneously with the specific antigen and vasoactive drugs (such as caffeine) all features and stages of allergic polyangitis could be induced by us (7 13, 14)

These cursory remarks are intended to show that the careful observations of J Waldenström provided a clinical counterpart to experimental findings and opened new avenues for clinical therapy on one hand and experimental work on the other We consider this typical of his work which enriched so many different fields of medicine

References

1 Bahrmann E Ueber ein gleichzeitiges Vorkommen von Asthma bronchiale mit rheumatischer eosinophiler Gefässentzündung Virchows Arch path Anat *296* 277 (1935)

2 Churg J and Strauss L Allergic granulomatosis allergic angitis and periarteritis nodosa Amer J Path *27* 277 (1951)

3 Hinson K F W Arteritis in asthma In "The nature of asthma" report of a Symposium held at King Edward VII Hospital Midhurst (England) May 1964

4 Kallos P Introduction Progr Allergy 4 1 (1954)

5 Kallos P and Kallos Deffner L Die experimentellen Grundlagen der Erkennung und Behandlung der allergischen Krankheiten Erg Hyg *19* 178 (1937)

6 Id Die experimentellen Grundlagen der Allergielehre Progr Allergy *1* 5 (1939)

7 Id Untersuchungen über die Tuberkulin Hautreaktion Acta med scand *109* 574 (1942)

8 Kallos P and Pagel W Experimentelle Untersuchungen über Asthma bronchiale Acta med scand 91 292 (1937)

9 Leopold J G A contrast of bronchitis and asthma In "The nature of asthma" report of a Symposium held at the King Edward VII Hospital Midhurst (England) May 1964

10 Löffler W Transient lung infiltrations with blood eosinophilia Int Arch Allergy 7 300 (1955)

11 Meyenburg H v Ueber eosinophile Lungenentzündungen Schweiz Z Tbk 1 705 (1944—45)

12 Noelpp Eschenhagen I and Noelpp B New contributions to experimental asthma Progr Allergy 4 361 (1954)

13 Pagel W Pathologie und Histologie der allergischen Erscheinungen Progr Allergy 1 43 (1939)

14 Pagel W and Treip C F Viscero cutaneous collagenosis J clin Path 8 1 (1955)

15 Waldenström J Some clinical aspects of the diagnosis and treatment of vascular allergy Int Arch Allergy 7 309 (1955)

Royal Northern Hospital London

Waldenstrom's Uveoparotitis

By D GERAINT JAMES
M A M D (Cantab) F R C P (London)

Thirty years ago Jan Waldenstrom (1) submitted for publication in Acta Medica Scandinavica an account of his observations of patients with uveoparotitis attending the medical surgical and ophthalmological clinics in Upsala He described five patients with bilateral parotid gland enlargement and bilateral uveitis Four of them were women and they were all over 30 years of age The disease process was clearly multisystem for one or other of these patients had evidence of involvement of the neurological system, lungs lymph nodes or skin as well as fever or hyperglobulinaemia Sarcoid tissue was clearly demonstrable in the parotid gland tissue of one patient Waldenstrom drew particular attention to bizarre neurological manifestations of sarcoidosis which included right sided facial palsy in two patients and a right hypoglossal nerve palsy or bilateral optic neuritis once apiece One patient

"was regarded as a case of encephalitis lethargica with signs of involvement of the brain stem (lethargy), the *cerebral* cortex (hallucinations) the pyramidal tract (positive Babinski reflexes), the sensory system (impaired deep sensibility in the toes and other disturbance of sensibility) the cerebellum (positive sign of Romberg intention tremor, signe de frein) and of the peripheral neurone (abolished patellar and achilles reflexes peripheral facial palsy) At the same time there was conclusive evidence of meningeal involvement (pleocytosis increase in albumen and pathological mastix curve)

The significance and importance of this publication can only be evaluated in the light of current thinking at that time Heerfordt (1909) (2) had already drawn attention to the syndrome of "Febris uveoparotidea subchronica characterised by uveitis and enlargement of the parotid glands running a chronic and usually febrile course and frequently complicated by cranial nerve palsies especially of the seventh cranial nerve with pleocytosis of the cerebrospinal fluid Heerfordt described three cases and referred to other examples he had found in the literature Since some of these latter

Some observations on uveoparotitis and allied conditions with special reference to the symptoms from the nervous system

by

JAN WALDENSTRÖM

A great number of cases of the so-called febris uveoparotidea Heerfordt has been published in ophthalmological journals and the syndrome is regarded as a question of interest [illegible]. The publications of recent years, however, have shown that [illegible] a pathological process [illegible] practically all the organs of the body. The malady thus deserves also the interest of the [illegible]. I am here [illegible] the following [illegible] a few cases from the medical, [illegible] and ophthalmological [illegible] that might illustrate the different [illegible] aspects of this curious syndrome.

Case 1. (The case has been published by Professor Bergmark in Svenska [illegible] medicinska förhandlingar 1933. Person [illegible] woman aged 52. Previous history of no interest. [illegible] children [illegible] good health. In 1933 [illegible] cholecystitis. Since then perfect health till March 15th 1933, when patient began to feel tired and feverish. She has been very sleepy and has only for short intervals been able to be out of bed. At times she has suffered from headaches, her appetite has decreased [illegible]. On March 23rd [illegible] red patches on [illegible] were noticed.

Admitted to the Medical Clinic March 26th [illegible] Patient [illegible] she falls asleep repeatedly during the [illegible]. She gives adequate answers to questions but [illegible] sleep [illegible].

[illegible]

Fig. 1. The first page in Acta Medica Scandinavica of Waldenström's classic paper on Uveoparotitis



Fig. 2. Waldenström's first patient with Uveoparotitis, erythema nodosum, optic neuritis and diffuse cerebral involvement. If she is still alive she is now aged 82 years.

examples were ascribed to mumps. Heerfordt was content that his cases were of similar aetiology. During the following quarter century the aetiology of *Heerfordt's syndrome* was fiercely debated. Two main schools of thought favoured either mumps or tuberculosis. This controversy ended when Waldenström produced compelling arguments identifying uveoparotitis as but another manifestation of sarcoidosis. His cogent reasons against the tuberculous aetiology were the negative tuberculin skin tests, the absence of caseation in the tissues and the inability to isolate tubercle bacilli from the lesions. He cited in favour of the identity of uveoparotitis and sarcoidosis the similarity of the skin lesions and of the histology and the occurrence in both of a similar hyper*globulinaemia*.

Those of us studying the problem one generation later can add nothing of value to Waldenström's masterly account of uveoparotitis and we can but admire the way in which he synthesised diverse clinico-pathological aspects under the single heading of sarcoidosis. Involvement of the parotid gland due to sarcoidosis is but one incident in a disease affecting many systems. The most certain way of segregating sarcoid parotitis from the se

veral causes of enlargement of the parotid gland is by seeking corollary evidence in other tissue systems and by obtaining histological proof of sarcoid tissue In a recent series (3) enlargement of the parotid gland was observed in 23 of 388 (6 %) patients with histologically-confirmed sarcoidosis The most frequent clinical accompaniments are enlargement of spleen and lymph nodes, uveitis and abnormal chest radiographs, whilst histological confirmation is most conveniently obtained by biopsy of lymph node skin liver, parotid or by means of the Kveim test Sarcoid parotitis may be acute, transient and self-limiting, or it may be chronic and persistent Corticosteroids seem unnecessary in the transient group and provide no clearcut benefit in chronic persistent enlargement of the parotid gland However, steroids have influenced the natural history of uveitis and are always indicated, either topically or parenterally, to control this otherwise distressing and crippling manifestation of uveoparotitis (4)

References

1 Waldenström J "Some observations on uveoparotitis and allied conditions with special reference to the symptoms from the nervous system" Acta med Scand 1937 *91* 53

2 Heerfordt C F "Febris Uveo parotidea subchronica" v Graefe s Arch Ophthal 1909 *70* 254

3 Greenberg G Anderson R Sharpstone P James D G "Enlargement of parotid gland due to sarcoidosis" Brit Med J 1964 2 861

4 James D G Anderson R Langley D Ainslie D "Ocular sarcoidosis" Brit J Ophthal 1964 *48* 461

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Radiovitamin B_{12} as a biological reference substance III
Glomerular filtration rate

By P O Granberg and Peter Reizenstein

Vitamin B_{12} is one of the few physiologically occurring organic molecules which can be labeled with a gamma emitting radio isotope without changing the molecule itself. The localization of the cobalt atom centrally in the cobalamin molecule provides for an extraordinary stability of the label. No liberation *in vivo* of the radio cobalt from the cobalamin has been demonstrated (1 2). In addition vitamin B_{12} can be labeled with four different radioactive cobalt isotopes and the substance is quite non toxic. No instances of hypersensitivity to vitamin B_{12} have been reported. These facts render radiovitamin B_{12} unusually suitable for the use as a biological reference substance in the physiological and chemical studies of different organs. The two first parts of this project involved the use of radiovitamin B_{12} quite successfully in the study of gastric function (3) and with rather less success in the study of hepatic function (4).

Supported by the Swedish Medical and Technical research councils and the J & T Andersson Foundation.

The purpose of the present work is to examine the possibility to use radio vitamin B_{12} in the study of renal function *i e* to compare glomerular filtration rates obtained with radio B_{12} to those obtained with inulin. The basis for this idea was the observation that renal disease affects the result of the urinary vitamin B_{12} excretion test in diagnostic studies of hematologic conditions (5).

During the progress of this work two articles have appeared (6 7) showing good agreement between the glomerular filtration rate measured with inulin and that measured with vitamin B_{12}. These results are confirmed and extended in the present paper.

Material and methods

Six patients were investigated and all together 34 clearance periods were studied with each method. The patients are described in table 1. The investigations were performed in the morning with patients in the fasting

Table I *Description of patients*

Symbol	Age/sex	Diagnosis	No of clearance periods
A C	57/f	Stenosis of renal artery with normal kidney function	6
KAB	20/m	Volunteer control with normal kidney function [1]	6
KG	40/m	Volunteer control Normal kidney function [1]	6
SEN	33/m	Volunteer control Normal kidney function [1]	6
OW	72/m	Renal carcinoma on right side	6
GGT	36/m	Stenosis of renal artery with hypertension	4

[1] *Patient treated with Octapressin®* (phenyl alanine lysine vasopressine) after 2 control *periods in the clearance study*

state The patients were hydrated be*fore and during the investigation The* intravenous infusions of the clearance substances were made through a percutaneously introduced polyethylene catheter in a brachial vein In order to saturate the B_{12} binding capacity of the serum, a loading dose of 2 000 μg *cyanocobalamin* was *given The specific activity was 0 4 nc/μg and the* ^{57}Co label was used Simultaneously, 0 5 ml of a 10 per cent inulin solution per kg body weight was given This prime dose was followed by an intravenous infusion with a constant speed syringe pump at a rate of 0 5 ml or 15 μg cyanocobalamin and 37 5 mg inulin per *minute* This infusion was given during a 30 minute equilibration period prior to the *first clearance period* and continued during the entire test

Plasma samples for determination of inulin and B_{12} *were collected in the* middle of each clearance period through a polyethylene catheter introduced in the brachial artery These periods lasted from 15—20 minutes

The bladder was evacuated with a *Foley* catheter *a demeure* To minimize the dead space factor, the emptying procedure at the end of the periods was assisted by flushing with water and air as described earlier (8)

Inulin determinations in plasma and urine were performed colorimetrically at 530 mμ as described (9)

Radioactivity determinations were performed on 3 ml plasma or urine samples in a 2 ½"×3" well type NaI (Th) crystal with the help of a Gam matrix sample changer Pulses amplified 250 times were recorded on an Ekco N 650 scaler pulse height analy*zer and measurement times so* adjusted, that the average and maximum *measurement errors secondary* to the random nature of the disintegrations were respectively 0 13 per cent and 0 71 per cent for urine and 2 7 per cent and 3 3 per cent for plasma

Results

The deviations from the ideal constant concentration in the serum of radio B_{12} and inulin respectively during the experimental period were studied and expressed as the mean of the standard deviation in per cent of the mean concentration for each individual patient These percentages were 4 99 for inulin *and 4 06 for* B_{12}

A statistically significant correlation

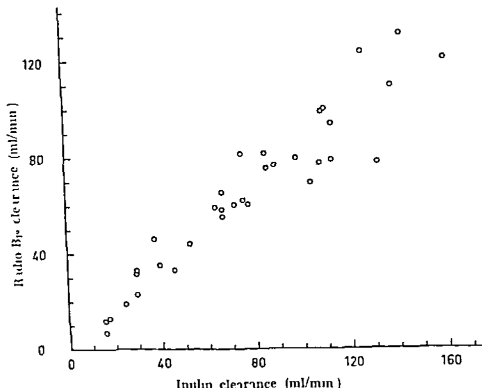


Fig 1 Correlation between glomerular filtration rate as measured with radio B_1 and with inulin

between the glomerular filtration rates determined with radio B_{12} and with inulin was found

The correlation coefficient was 0 95 and the regression line for B_1 upon inulin was

$$B_1 = 5\ 155 + 0\ 764\ (\text{inul})$$

The S D of the regression coefficient was 0 0442 and the regression coefficient thus differs from zero in a statistically significant manner ($p<0\ 01$)

Discussion

Glomerular filtration rates are usually measured with the inulin clearance method Inulin is filtered in the glomeruli and neither secreted nor reabsorbed in the tubules However the customary colorimetric inulin determinations are rather laborious Another disadvantage is that both glucose fructose and certain drugs such as dextrane interfere with the inulin assay

All these circumstances have made inulin clearance determinations laborious difficult and rare In clinical routine the endogenous creatinine clearance is therefore used to measure the glomerular filtration rate However the values thus obtained are ap

proximate since there is both secretion and reabsorption of creatinine in the tubules This renders the clearance values obtained with creatinine falsely too high in, *e g*, uremic conditions

It is our impression that radiovitamin B_{12} has none of the disadvantages of inulin, and might provide a method of choice for determining the glomerular filtration rate

Summary

Vitamin B_{12} being a physiologically occurring organic compound with a gamma emitting isotope as a stable, natural part of the molecule, is suitable as a biological reference substance In parallell studies of the glomerular filtration rate with inulin and radio B_{12}, a statistically highly significant correlation with a coefficient of 0,95 was found Radio B_{12} determinations had smaller errors than those of inulin and were much less laborious Substances interfering with inulin assays do not interfere with the assays of radio B_{12} The serum concentrations of inulin were less constant than those of radio B_{12} which might provide a method of choice to measure the glomerular filtration rate

References

1 Rosenblum C Reizenstein P Cronkite E P Meriwether H T Tissue Distribution and Storage Forms of vitamin B_{12} injected and orally administered to the dog Proc Soc Exp Biol Med *112* 262 1963

2 Reizenstein P Kulsdom N Conversion of cyanocobalamin to a physiologically occurring form Blood in press

3 Schutz H B Reizenstein P Radiovitamin B_{12} as a dilution indicator in gastrointestinal research Am J Dig Dis *8* 904 1963

4 Göthe C J Influence of Liver Function on the Excretion of vitamin B_{12} Scand J Clin Lab Invest *17* 99 1965

5 Reizenstein P Diagnosis of pernicious anemia with radio B_{12} (in Swedish) Sw Soc Int Med Jan 25 1957 (Nord Med *61* 65 1959)

6 Breckenridge A Metcalfe Gibson A Methods of measuring glomerular filtration rate Lancet *ii* 265 1965

7 Slapak M Hume D M A new Method of Estimating Glomerular filtration Rate Lancet *i* 1095 1965

8 Granberg P O Effect of acute hypoxia on renal hemodynamics and water diuresis in man Scand J Clin Lab Invest suppl 63 1962

9 Heyrovsky A A new method for the determination of inulin in plasma and urine Clin Chim Acta *1* 470 1956

From the Medical Department II St Erik s Hospital Stockholm
(Head Professor Bengt J E Ihre)

Pressure variations in the rectum and ileum during experimentally induced urgency of defecation

By Franz Barany

One occasionally encounters diarrhea of obscure causation This condition is usually classified as "functional diarrhea" and the course of the disease seems often to be influenced by psychological factors The present study was undertaken to examine whether in such cases the mechanism of defecation presents any abnormality and if so to analyse it

The series

The following groups of patients were chosen for this investigation

14 control subjects evacuating once daily without discomfort the stools being normal in shape and consistency

4 patients with constipation who had not *more than 2 spontaneous defecations* a week They had been instructed *not* to use laxatives for at least 3 days before the experiment

11 patients with functional diarrhea diagnosed at this department all having at least 4 loose stools a day one or more with imperative urgency No antidiarrheal drugs had been given for at least 2 days *before the experiment* Achlorhydria steatorrhea pancreatic insufficiency enteritis colitis and intestinal stenosis and tumour had been excluded by thorough clinical and radiological examinations

11 patients who at least one year before the experiment had undergone partial gastrectomy and Polya gastroenterostomy 7 of them also bilateral truncal vagotomy Two of the vagotomy patients had occasional diarrhea at irregular intervals and seldom lasting more than a day Otherwise the patients in this group had 1—3 evacuations a day with stools of normal or almost normal consistency None had diarrhea during the period of the experiment

6 patients who *at least* one year before the experiment had had a partial resection of the rectum and/or colon with an end to end anastomosis All had *suffered* from carcinoma of the bowel but at the time of the experiment were feeling well and there were no signs of metastases

proximate since there is both secretion and reabsorption of creatinine in the tubules This renders the clearance values obtained with creatinine falsely too high in, *e g*, uremic conditions

It is our impression that radiovitamin B_{12} has none of the disadvantages of inulin, and might provide a method of choice for determining the glomerular filtration rate

Summary

Vitamin B_{12} being a physiologically occurring organic compound with a gamma emitting isotope as a stable, natural part of the molecule is suitable as a biological reference substance In parallell studies of the glomerular filtration rate with inulin and radio B_{12}, a statistically highly significant correlation with a coefficient of 0,95 was found Radio B_{12} determinations had smaller errors than those of inulin, and were much less laborious Substances interfering with inulin assays do not interfere with the assays of radio B_{12} The serum concentrations of inulin were less constant than those of radio B_{12} which might provide a method of choice to measure the glomerular filtration rate

References

1 Rosenblum C Reizenstein P Cronkite E P Meriwether H T Tissue Distribution and Storage Forms of vitamin B_{12} injected and orally administered to the dog Proc Soc Exp Biol Med *112* 262 1963
2 Reizenstein P Kulsdom N Conversion of cyanocobalamin to a physiologically occurring form Blood in press
3 Schutz H B Reizenstein P Radiovitamin B_{12} as a dilution indicator in gastrointestinal research Am J Dig Dis *8* 904 1963
4 Göthe C J Influence of Liver Function on the Excretion of vitamin B_{12} Scand J Clin Lab Invest *17* 99 1965
5 Reizenstein P Diagnosis of pernicious anemia with radio B_{12} (in Swedish) Sw Soc Int Med Jan 25 1957 (Nord Med *61* 65 1959)
6 Breckenridge A Metcalfe Gibson A Methods of measuring glomerular filtration rate Lancet *ii* 265 1965
7 Slapak M Hume D M A new Method of Estimating Glomerular filtration Rate Lancet *i* 1095 1965
8 Granberg P O Effect of acute hypoxia on renal hemodynamics and water diuresis in man Scand J Clin Lab Invest suppl 63 1962
9 Heyrovsky A A new method for the determination of inulin in plasma and urine Clin Chim Acta *1* 470 1956

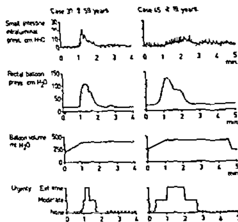


Fig 1 Controls Urgency and contraction of rectum provoked by filling the rectal balloon they disappeared spontaneously although the balloon volume was kept constant Pressure activity of small intestine synchronous with rectal contraction

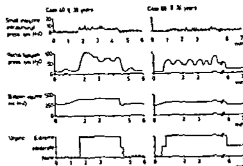


Fig 2 Functional diarrhea No spontaneous disappearance of urgency or rectal contraction unless the balloon volume was reduced Pressure activity of small intestine synchronous with rectal contraction

15—25 ml to the balloon or to decrease the volume by 25—50 ml and then refill by the same amount

The attacks of urgency had no regular effect on the propulsion of the endoradiosonde In some instances its position was unchanged throughout the procedure while in others the sonde moved considerable distances before during and after an attack

The 4 *constipated* patients displayed no deviation from the normal course

In all the cases of *functional diarrhea* the rectum showed an inability to adapt itself to a filling that had provoked contraction There was either an apparently tonic contraction of the rectum or a succession of rhythmic contractions and abortive relaxations In neither case did the feeling of urgency or the provoked activity of the small intestine disappear unless the volume of the rectal balloon was reduced The reactions of 2 cases of functional diarrhea are illustrated in figure 2

In the 7 patients that had had a *partial gastrectomy Polya gastroenterostomy and truncal vagotomy* filling elicited a normal contraction of the rectum and feeling of urgency One of them exhibited normal adaptation almost complete relaxation being recorded within 2 minutes In the others relaxation was slower taking more than 2 minutes during which several pressure waves of decreasing height were recorded (Figure 3) In 3 patients no pressure activity of the ileum was provoked during the attack of urgency In 2 there was hardly any evidence of such activity Only in the remaining 2 patients was a normal and distinct response of the small intestine obtained

A completely normal response was found in 3 of the 4 subjects that had had a *Polya operation without truncal*

Methods

The intestinal motility was recorded by the endoradiosonde technique The endoradiosonde Type P 2 (5) is sensitive to intraluminal pressure and transmits information to a receiving system outside the patient The sonde is 9 mm in diameter 16 mm long and weighs 2 3 g The intraluminal pressure waves are plotted by an ink recorder The movements of the endoradiosonde through the small intestine are charted by a tracking antenna system (4) The patient lies on a couch and a sheet of methacrylate 60×64 cm slightly curved and covered with a sheet of paper is placed a few centimeters above the abdomen The movements of the antenna which follows the sonde are recorded by an ink pen on the paper

The sonde was swallowed by the gastroenterostomy patients immediately before breakfast at 8 30 a m and by the other subjects 2—4 hours before The experiment was started at 9 a m or later when the sonde had reached the middle or lower part of the small intestine The position of the sonde was judged from the chart drawn by the antenna system

A thin walled rubber balloon was introduced into the rectal ampulla and filled with water at 37° C until the patient felt urgency The balloon had a neck 6 cm long and 1 2 cm in diameter the axial length of the ovoid body of the balloon was 14 5 cm Its inflated diameter undistended was 11 cm The neck of the balloon was tied around a plastic catheter which was used for filling and pressure recording and also served as a support when the collapsed body of the balloon was to be inserted into the rectal ampulla The filling was performed slowly in increments of about 25 ml with intervals for recording As a rule it took about 10 minutes to reach the urgency threshold

During urgency the pressure in the balloon rose steeply The pressure can be considered equal to the rectal pressure since the capacity of the undistended balloon was always greater than the volume of water used Several attacks of urgency were produced in each patient by varying the water volume The intraluminal ileal pressure and the movements of the endoradiosonde were recorded continuously In analysing the pressure curves the classification of Code Hightower & Morlock (2) was used

Results

The course of events when the rectal balloon was filled slowly in a normal subject was as illustrated in figure 1 At a volume of between 225 and 500 ml the rectum suddenly contracted so that the balloon pressure rose from a reference value of 5—15 cm to 45—130 cm H_2O Before this contraction the patient had experienced only a sensation of filling in the rectum Simultaneously with the contraction, however, urgency and slight discomfort was felt, which was described as arising in the upper part of the abdomen as well as in the rectum At the same time there was a burst of pressure waves from the small intestine consisting either of several type I waves or of a single type III wave The patient was asked to avoid straining to prevent interfering pressure waves

The filling of the balloon was always stopped at the onset of urgency, and in the control subjects the rectum then became adapted within 2 minutes the balloon pressure falling to the reference value, the feeling of urgency disappearing and the pressure activity of the small intestine returning to normal When relaxation was complete the normal rectum did not contract again even though the balloon volume was not changed for 5—8 minutes To provoke a new attack of urgency it was necessary either to add

less than 2 minutes starting and ending at the reference line and accompanied by urgency The individual contraction seemed not to differ from that for the controls where however it was not repeated In none of the patients of this group was there any effect on the ileal pressure activity (Figure 4)

Two patients had undergone a *partial resection of the colon* for carcinoma and an end to end anastomosis had been performed in one of them between the proximal part of the transverse colon and the descending colon and in the other between the proximal part of the descending and the sigmoid colon In the experiment these patients reacted as normal subjects in every respect

Discussion

The recto ileal reflex described by Barany & Jacobson in a preliminary communication (1) seems to have its trigger zone in the upper part of the rectum or at the rectosigmoidal junction In patients who had had this part of the bowel resected no motility of the small intestine could be elicited by distending the remaining adjacent parts

The fact that only 2 of the 7 patients with truncal vagotomy had a normal reflex suggests that a vagal branch is the efferent pathway

Using fluoroscopy *Velho da Silva* observed a rectocaecal reflex which implied a contraction of the caecum and ascending colon as a result of rectal distension (6) Connell Frankel and Guttman found that distension of the rectum produced a pressure activity of the sigmoid colon which they suggested had the effect of retaining bowel contents in the colon while the rectum was being emptied (3) A study of the movements of the endoradiosonde during attacks of urgency with and without synchronous pressure activity of the small intestine did not reveal any tendency for the recto ileal reflex to inhibit propulsion

It seems justified for the subsequent discussion to assume that the recto ileal reflex is part of a more generalized activation of the bowel elicited by distension of the trigger zone Since vagotomy can abolish the ileal response the activation would seem in some measure to depend upon vagal impulses Other efferent nerves must however also be involved especially those to the transverse left and sigmoid colon This could explain why patients that have undergone vagotomy and those with resection of the trigger zone both lack the recto ileal reflex while only the former assign part of the urgency to the upper abdomen

The persistent relaxation of the rectum which normally follows within 2 minutes of a contraction *provoked* by distension implies adaptation to the distension and is essential for normal bowel habits If the adaptation *does* not take place there will soon be an urgency to defecate irrespective of the time of day or of the number of *previous* bowel movements that day Such a lack of adaptation to distension was found in all the cases of

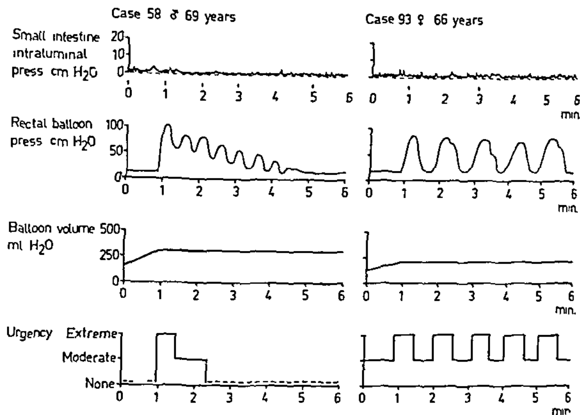


Fig 3

Fig 4

Fig 3 Polya resection with vagotomy Prolonged period of rectal relaxation with pressure waves of decreasing amplitude No pressure activity of the small intestine provoked by attack of urgency

Fig 4 Proximal rectum resected Constant distension of bowel provoked a series of pressure waves Complete relaxation between waves No pressure activity of the small intestine corresponding to bowel contractions

vagotomy The remaining patient showed a few rhythmic contractions of the rectum during the relaxation period, which however still terminated within the normal 2 minutes

Four patients had undergone *resection of the upper part of the rectum* for carcinoma and the anastomosis to the sigmoid was located 6—10 cm from the anus The balloon volume necessary to produce contraction of the bowel was *slightly less (175—215 ml)* in these patients than in the controls The contraction was accompanied by a feeling of urgency but this was *felt only in the rectal region and* not in the upper part of the abdomen as was the case in all other groups Pressure activity of the small intestine synchronously with rectal contraction was not seen in this group *The bowel* of these patients showed a remarkable activity as recorded from the balloon thus without any change of the balloon volume a seemingly unlimited *series of pressure waves of fairly* constant amplitude was obtained The contractions occurred at intervals of

less than 2 minutes starting and ending at the reference line and accompanied by urgency The individual contraction seemed not to differ from that for the controls where however it was not repeated In none of the patients of this group was there any effect on the ileal pressure activity (Figure 4)

Two patients had undergone a *partial resection of the colon* for carcinoma and an end to end anastomosis had been performed in one of them between the proximal part of the transverse colon and the descending colon and in the other between the proximal part of the descending and the sigmoid colon In the experiment these patients reacted as normal subjects in every respect

Discussion

The recto ileal reflex described by Barany & Jacobson in a preliminary communication (1) seems to have its trigger zone in the upper part of the rectum or at the rectosigmoidal junction In patients who had had this part of the bowel resected no motility of the small intestine could be elicited by distending the remaining adjacent parts

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The persistent relaxation of the rectum which normally follows within 2 minutes of a contraction provoked by distension implies adaptation to the distension and is essential for normal bowel habits If the adaptation does not take place there will soon be an urgency to defecate irrespective of the time of day or of the number of previous bowel movements that day Such a lack of adaptation to distension was found in all the cases of

functional diarrhea and in the patients who had undergone resection of the proximal part of the rectum and the rectosigmoidal junction Furthermore, most of the vagotomized patients showed a slower adaptive relaxation of the rectum than the controls

A comparison of these three groups indicates that the trigger zone in the rectum has two functions It activates the gut through reflexes, which are partly vagal, and it is the origin of a slow inhibitory mechanism, which normally extinguishes the rectal contraction and the reflexes within 2 minutes

In the cases of functional diarrhea there was activation but no inhibition Distension of the rectum provoked an almost tonic contraction and a persistent recto ileal reflex Urgency was felt in the upper abdomen and soon became unbearable The question is whether the hyperirritability' of the gut in these patients is due to a local disturbance of the trigger zone or to a more generalized malfunction In this connection it should be mentioned that the distension threshold for contraction was not obviously lower in the diarrhea patients than in the controls

In patients where the trigger zone had been resected there was no evidence of activation or inhibition The distension elicited an unlimited series of local pressure waves but no recto ileal reflex, and each wave was followed by complete relaxation Urgency and discomfort were much less marked than in the diarrhea patients and were not felt in the upper abdomen The resection patients did not suffer from diarrhea but noticed a change in their bowel habits after the operation They could no longer withstand a call to stool for more than a short period, and they usually had an evacuation after each meal

In the vagotomized patients the activation was often partly eliminated, and then no recto ileal reflex was recorded, but urgency was still felt in the upper abdomen The inhibition may also have been affected to some extent, since rectal adaptation was delayed The clinical significance of these changes remains to be established

Summary

The rectum of 14 healthy controls and 32 patients with various gastrointestinal disturbances was subjected to gradual distension by means of a water filled balloon Ileal motility was recorded with an endoradiosonde and rectal motility was recorded from the balloon

Distension elicited a recto ileal reflex, except in 5 out of 7 patients that had undergone truncal vagotomy and in 4 with resection of the upper rectum

Adaptation of the rectum to distension was normally complete within 2 minutes It was absent in all 11 cases of functional diarrhea and in the 4 rectal resection patients There was a marked difference between these two groups The cases of functional diarrhea showed an almost tonic contraction of the rectum which was accompanied by a persistent recto ileal reflex

during distension Urgency soon became unbearable

In the resection group the distension provoked a series of rhythmic contractions and relaxations but no recto ileal reflex Urgency was much better tolerated and there was no diarrhea

References

1 Barány F & Jacobson B (1964) 7e Congres Internationale de Gastro Enterologie Bruxelles

2 Code C. F Hightower N C Jr & Morlock C G (1952) Amer J Med 13 328—351

3 Connell A M., Frankel H & Guttman L (1963) Paraplegia 1 98—115

4 Jacobson B & Lindberg B (1963) Electronics 36 58—60

5 Jacobson B & Nordberg L (1961) IRE Trans bio med Electron., BME—8 192—196

6 Velho da Silva J J (1948) Rev Médico Cir Brasil 56 29—65

From the Department of Internal Medicine Serafimerlasarettet Stockholm Sweden (Head Prof G Biörck)

The three patients

By Gunnar Biörck

During the years 1950—1958 I had the privilege to work under and with Jan Waldenstrom in Malmo In this period, carcinoids, heart surgery and the epidemiology of atherosclerosis all presented challenges to the cardiologists of his medical department One of the areas, into which we had to expand was the automatic processing of information from many thousands of hospital records concerning patients with rheumatic fever valvular heart disease and myocardial infarction Experiences from our work in Malmo were fundamental to us when P Hall and I during the 1960 s were forced into the creation and development of a center for automatic processing of medical records in my department of medicine at Serafimerlasarettet Stockholm

The handling of hospital records by data technique is not only and not mainly, a technical problem The challenge is primarily a medical one to the physician who has to define what the significance of the record is in *his method of dealing* with the patient The problem thus, is one of describing in terms of operational analysis, how the physician works and at the same time to identify the structure, and substructures, of a hospital record For not only must the record make sense to the doctor, it must at the same time be construed as to make sense to a data machine Such endeavours may appear somewhat painful to the clinician for whom so much of this has been imbibed into his central nervous system as to have assumed the quality of intuition rather than technique

I shall not venture into philosophy but I think it is fair to state that there are always *two* patients the *real* one and the *image* of him in the physician's mind (Fig 1) But in *hospital* medicine there are always at least *two imaginary* patients for every real one the image in the doctor s mind and the *patient-on record* The patient on record is at times more informed and more informative than the real one (although occasionally he will tell us as much about the author of the record as of the patient) In most cases the patient on record and the real patient differ in composition (Fig 2) in some respects the record

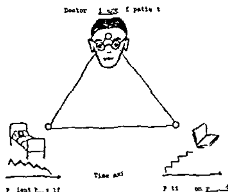


Fig 1

goes deeper under the surface as is true of data on earlier disease which the patient himself may have forgotten about or of laboratory data — in other respects the patient may be hiding important information below the surface making it unaccessible for the record which I believe is particularly true of psychological data

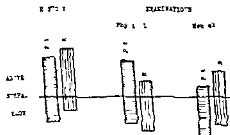


Fig 2

In his work the physician operates with all the three versions of the patient the real patient the patient on record and the image in the physician's mind which consists of the other two mixed and distorted by the physician's own temperament

Fig 3

PHYSICIAN

PATIENT RECORD

Physician compares

PATIENT ⇄ IMAGE
PATIENT ⇄ RECORD
RECORD ⇄ IMAGE

A hospitalized patient proceeds along a time axis and the physician tries to keep his image of the patient up to date by comparing impressions from the real patient with information contained in the record (Fig 3) There is to day an appreciable tendency among junior doctors to study the record closer than the patient This is a problem which merits a paper by itself

A few words might be added concerning the production of the medical record In a quantitatively limited medical practice the physician might even do without records relying only on his own memory However in our country a law has recently made record writing obligatory for all physicians who treat patients With greater patient loads and a more sophisticated technology the record writing may develop along various lines as seen in fig 4 It is interesting to note that in the case of the medical history the sequence may be radically changed through active participation by the patient at least to a *certain* extent (selfadministered questionnaire) It is also worth remembering that *many pieces of information* such as laboratory data consultant *opinions etc* may be entered in the record without

Fig 4

PHYSICIAN

Information → own memory store
Information → own memory store → own handwritten record
Information → own memory store → secretary → typewritten record
Information → own memory store → dictaphone → secretary → typewritten record
Information → own memory store → dictaphone → secretary → punch tape → magnetic memory → automatically typewritten record

SELF ADMINISTERED QUESTIONNAIRE

Information → secretary → punch tape → magnetic memory → automatically typewritten record → *physician* → own memory store

passing first through the attending physician

Now, how does the doctor *work* with the real patient, the patient on record and the image of the patient? Figure 5 represents an attempt to indicate, briefly and inadequately, the various levels at which activities oc

Fig 5

	DOCTOR—PATIENT	DOCTOR S IMAGE	RECORD
1	Take history Explain calm	Concept of patient	Notes
2	Examine Alleviate pain		Notes
3			Assort notes
4		"Case"	Write record
5		Interprete	
6		Analyse possibilities	
7		Conclude diagnosis (If insufficient *evidence* Repeat)	Record
8	Further examinations		Add new data
9		Final diagnosis	Record
10	Inform patient		
11		Decide on action	Record
12	Treat		Record treatment
13	Observe effects		Record effects
14		Evaluate treatment	
15	Inform advise		Summarize
16		Remember — or forget	Store

cur I believe it demonstrates that the record is an indispensable instrument by means of which the running medical decision making is made possible at the same time as the decisions and the basis upon which they are founded are codified

The fact that disease in and care of a patient is a dynamic process and not a static situation represents one of the big difficulties in collecting and recording of medical data Most information on a patient is obtained pointwise and only rarely continually — with the exception of continuous monitoring of cardiovascular and similar parameters in some intensive care units The more careful or even continuous recordings of patient data along a time axis are the more bulky becomes the collected material the more difficult to grasp for the physician and the more imperative becomes the need of concentration and the boiling down of the material to essentials In as much as an increasing percentage of our patients are older people often with a loaded medical history with many more recorded examinations and episodes of disease than formerly with more treatment and medication and — perhaps — with a more extensive interplay of medical psychological and social factors than hitherto recognized the medical record of to day is doomed to become a more comprehensive but less digestible document than in earlier times Certainly this is one of the big problems before us The data machine can reduce the storage space to a few centimeters on a magnetic tape but it cannot by itself condense the material into a truly representative and useful summary

We know from the experience of others and ourselves that the data machine to day can accumulate store and reproduce the alpha numerical information contained in the conventional medical record This mere fact does not tell us whether a datamachine produced medical record is better or worse than a conventional one But it does imply that the problem of record *storage* is solved and that *statistical analyses* of various kinds are greatly facilitated It will also facilitate the production of *check off lists* for future follow up studies of patient materials

However it is but natural that we are asking ourselves what possibilities with regard to the record proper are inherent in present data techniques What ways and means do we have to *increase* the *amount* of *useful information* in the individual patient and to make this information *more precise*? We know that there is much to be gained by *standardization* of questions and procedures — but we do not know what might be lost by the rigidity imposed by such methods (fig 6)

Above all we want to know whether the data technique will eventually offer us in addition to *information* also *interpretation diagnostic alternatives* and *recommendations* for *supplementary examinations* as well as for *therapy* in case of established diagnosis or a *preferential sequence* of therapeutic trials in case of *undecided* diag

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PHYSICIAN

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4		"Case"	Write record
5		Interprete	
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7		Conclude diagnosis (If insufficient evidence Repeat)	Record
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9		Final diagnosis	Record
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13	Observe effects		Record effects
14		Evaluate treatment	
15	Inform advise		Summarize
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Acknowledgements

Generous financial support to this special volume is gratefully acknowledged from contributors listed below

Grune & Stratton Inc New York
Nordisk Insulinlaboratorium Kopenhamn
The Scandia Group of Insurance Companies
AB Astra
AB Bofors Nobelkrut
AB Draco
AB Ferrosan
AB Hassle
AB Kabi
AB Leo
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Squibb AB
UCB Nordiska AB
Winthrop AB

In the same sense of congratulation a great number of Swedish collegues have paid homage to Jan Waldenström on his birthday by sponsoring this volume Their names will be found in a special adress of greeting which will be handed over to the celebrator on April 17 1966

Fig 6

INFORMATION

Accumulated factual data
(history, examination laboratory)

INTERPRETATION

Analysis of possible diagnoses establish priority order

RECOMMENDATIONS

1 DIAGNOSTIC→supplementary examinations
2 THERAPEUTIC
a) Diagnosis established — treatment of choice
b) Diagnosis *not* established — preferential sequence of trials

nosis This might appear to be easily within reach, but people often forget that clinical medicine is not an absolute science, many of its units and measures are arbitrary and subjective, with equally arbitrary and subjective dividing lines between normal and abnormal along a continuity, and — in addition to that — patients often have more than one disease at the same time To which one of an acute pancreatitis an abstinence delirium or a bronchopneumonia occurring simultaneously in a patient *should* we ascribe an elevated body temperature? It might also be of interest to investigate, whether the prevailing diagnostic concepts, as they have emerged through centuries, still with an easily discernible foundation in gross macroscopic pathology, might profitably be moulded upon other conceptual patterns

References

1 G Biorck Några synpunkter på diagnostikens fysiologi Sv Läkartidningen 60 311 1963
2 G Biorck Journalens uppgifter och utformning Sv Läkartidningen 61 1309 1964
3 G Biorck Sjukjournalen ar instrumentet i den medicinska beslutsprocessen Lakartidningen 62 746 1965
4 G Biörck and O Engkvist Sjukhusjournalen Sammanfattning Lakartidningen 62 2937 1965
5 G Biorck Läkarens yrke Lakartidningen 62 3560 1965
6 P Hall O Engkvist C Mellner and T Danielsson Patienten och datamaskinen Folksams hälsoråds faktaserie nr 1 1965

ACTA MEDICA
SCANDINAVICA

SUPPLEMENTUM 446

STUDIES ON HUMAN SERUM VERY-LOW-DENSITY-LIPOPROTEINS

BY
ANDERS GUSTAFSON

ACCOMPANIES VOL. 179

GÖTEBORG 1966

LP the method of determining and expressing protein content has often been a matter of discussion In the present investigation a comparative study was performed on washed and unwashed representative subfractions of the VLD-LP and fresh standards, human albumin The amount of protein was generally determined by the method of Lowry et al (53) As this method measures only the tyrosine content of the protein it was compared with the biuret method The concentration of the protein was also determined as 6 25 times the Kjeldahl nitrogen Good agreement was obtained between the three methods, suggesting that the tyrosine content of the VLD LP proteins and the human albumin was the same Therefore, no corrections have been made

In Table A the composition was recalculated as mole lipids and amino acid residues per g molecular weight of LP Composition data and LP molecular weights were obtained from Paper I The data used for molecular weights of lipid components and amino acid residues are given in the table

For added convenience in the comparison of the molar composition of different subfractions the lipid mole ratio has also been expressed per 100 amino acid residues (Table B) From data in this table certain similarities in the lipid protein composition are apparent The chylomicron fractions , fractions A and B agree well in molar as well as in per cent composition (Table IV) Fractions D, E and β-LP (data of Oncley, 1963) (50) reveal, when calculated by their mole lipid ratios, an interesting relationship The decrease in relative triglyceride content concomitant with increase in LP density is, of course, to be expected

The cholesterol ester content showed the greatest variation within an individual fraction It seems established that

Table A *Lipid and protein composition of five VLD lipoprotein fractions and the β-lipoproteins Data expressed as mole lipids and amino acid residues per g mol wt of lipoprotein*

Lipoprotein Fraction		Mole Lipids and Amino Acid Residues per g Mol Wt. of Lipoprotein				
	Mol Wt ($\times 10^6$)	FC	CE	PL	TG	PR
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β-LP*)	2.3	470	1300	670	280	4100

FC = Free cholesterol (Mol wt 387) CE = Cholesterol ester (Cholesteryl stearate Mol. wt. 654)
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Based on these experiences a standardized procedure was outlined for the isolation and purification of five arbitrarily chosen VLD-LP subfractions A, B C, D and E at centrifugal force 0 1 × 10⁶, 0 6 × 10⁶, 4 8 × 10⁶ 12 6 × 10⁶, and 139 × 10⁶ g min, respectively As much as 45-50% of the original lipid content of a subfraction was lost by the washings. Subjects with hyperglyceridemia which had been characterized previously were therefore used as donors to allow sufficient amounts of LP to be recovered and studied.

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BY

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The present thesis is based on the following publications

I Studies of the composition and structure of serum lipoproteins
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Biochemistry 4 596, 1965

II A new method for partial delipidization of serum lipoproteins
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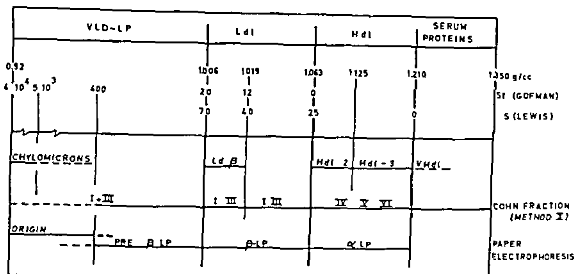


Figure A The serum LP spectrum LP designation by ultracentrifugal and electrophoretical methods and their relationship to hydrated density, Sf value (according to Gofman) - S value (according to Lewis) and to fractions of the isolation method by Cohn et al, Method X (13) The figure is based mainly on data collected by Cornwell and Kruger (20)

major somewhat overlapping LP classes have been identified within D 0 92—1 21 (Figure A), viz the chylomicrons, the very-low-density (VLD) LP, the low--density LP (Ldl) and the high-density LP (Hdl) Each of the four classes has been assumed to have its characteristic protein moieties, metabolism and function

The chylomicrons (24), primary particles (25) or alimentary particles (26) are large, light-scattering fat particles of alimentary origin, that normally appear in serum after a meal containing fat The problem of separating the chylomicrons from the VLD-LP *per se* has not yet been completely solved The LP of this heterogeneous class have in common the characteristic that they float in the ultracentrifugal field at D 1 006 The major portion of these triglyceride-rich and protein-low LP migrate electrophoretically with the α_2-globulins, or as a "pre-β fraction, α_2- or pre-β-lipoproteins (Figure A) Metabolically these VLD-LP seem to be involved in the transport of endogenous glycerides (27)

The low-density LP (Ldl), also named the β-LP from their electrophoretic mobility, is quantitatively the largest LP class in man and also the major cholesterol-bearing LP These LP are possibly related metabolically to the VLD-LP (28)

The protein and phospholipid-rich high-density LP (Hdl) or α-LP float at ultracentrifugation between D 1 063—1 21 (Figure A) The high-density LP can be subfractionated into Hdl-2 and Hdl-3 (29) having different lipid-protein ratios and physical characteristics but an identical protein moiety (30) and possibly identical physiological function In the serum residue at D 1 21 i e the infranatant at solvent density 1 21 g/cc a phospholipid-protein complex, called

the very high density LP (VHdl) has been identified (31, 32) containing a protein moiety identical to that of the α LP (33, 34) With the LP classes is often also included the free fatty acid (FFA) -albumin complex in the infranatant at D 1 21

The terms hypercholesterolemia, hyperlipemia and hyperlipidemia have been used to characterize disease entities with an elevation in one or more lipid components of the serum With the introduction of LP fractionation methods it has been apparent that the serum lipid changes reflect variations in amount of LP rather than changes in the composition of these LP It has therefore been found more appropriate to evaluate the serum lipid variations in terms of LP changes

The characteristic feature of essential hyperlipemia is an abnormal rise in serum triglyceride, in turn due to an increase in triglyceride-rich VLD LP Several clinical types of hyperglyceridemia occur (Chapter III)

The term essential hypercholesterolemia indicates a higher than normal amount of cholesterol in the serum, reflecting an increase in cholesterol rich β LP (hyper β lipoproteinemia) A disease entity with a combined hyper lipoproteinemia elevated VLD LP and β LP has been observed (see Chapter III) So far no cases of essential hyper-α lipoproteinemia have been reported

Congenital deficiency of a lipoprotein class a lipoproteinemia has recently been described for both β LP and α LP Lack of β LP, a-β-lipoproteinemia, Bassen-Kornzweig syndrome (35, 36) has clinically a malignant course with among other signs a defective exogenous fat transport system The a-α lipoproteinemia, the Tangier disease (37, 38), on the other hand causes only a mild reticuloendotheliosis and has an apparently normal chylomicron production

The research on serum lipids and LP was stimulated tremendously when in the early fifties it was suggested (39) that there might well be a relationship between increased levels of serum lipids and early coronary heart disease Different lipids and lipoprotein classes have been accused of atherogenic properties during the successive development in the knowledge of lipids Serum cholesterol (40) serum triglyceride (41, 42 43), serum cholesterol and triglyceride (44) β LP (Sf 10 20) (39) VLD LP (Sf 20 100) (45 46) and combinations of these parameters (atherogenic index) (47) all have been suggested predictors of subclinical arterial disease

Even if now a multifactoral cause for atherosclerosis has been established (48) the important participation of serum lipids and lipoproteins in this process can hardly be denied

This study was initiated by the finding of Furman et al, (49) of a high density LP being released from VLD LP by sonic forces The purpose of the present investigation was to characterize the complex class of VLD LP, including the chylomicrons and ultimately their protein moieties

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Based on these experiences a standardized procedure was outlined for the isolation and purification of five arbitrarily chosen VLD-LP subfractions A, B, C, D, and E at centrifugal force $0 1 \times 10^6$, $0 6 \times 10^6$, $4 8 \times 10^6$ $12 6 \times 10^6$, and 139×10^6 g min, respectively As much as 45-50% of the original lipid content of a subfraction was lost by the washings Subjects with hyperglyceridemia, which had been characterized previously, were therefore used as donors to allow sufficient amounts of LP to be recovered and studied

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LP the method of determining and expressing protein content has often been a matter of discussion In the present investigation a comparative study was performed on washed and unwashed representative subfractions of the VLD LP and fresh standards human albumin The amount of protein was generally determined by the method of Lowry et al (53) As this method measures only the tyrosine content of the protein it was compared with the biuret method The concentration of the protein was also determined as 6 25 times the Kjeldahl nitrogen Good agreement was obtained between the three methods suggesting that the tyrosine content of the VLD LP proteins and the human albumin was the same Therefore, no corrections have been made

In Table A the composition was re calculated as mole lipids and amino acid residues per g molecular weight of LP Composition data and LP molecular weights were obtained from Paper I The data used for molecular weights of lipid components and amino acid residues are given in the table

For added convenience in the com parison of the molar composition of different subfractions, the lipid mole ratio has also been expressed per 100 amino acid residues (Table B) From data in this table certain similarities in the lipid protein composition are apparent The chylomicron fractions', fractions A and B, agree well in molar as well as in per cent composition (Table IV) Fractions D, E and β LP (data of Oncley 1963) (50) reveal, when cal culated by their mole lipid ratios, an interesting relationship The decrease in relative triglyceride content concomitant with increase in LP density is, of course, to be expected

The cholesterol ester content showed the greatest variation within an indivi dual fraction It seems established that

Table A Lipid and protein composition of five VLD-lipoprotein fractions and the β-lipoproteins Data expressed as mole lipids and amino acid residues per g mol wt of lipoprotein

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Inconsistency in density of human sera made it necessary in the isolation steps to layer over the original serum sample and subsequent infranatants with a column of buffer solution, D 1 006 The procedure of overlayering has been used previously in the isolation of chylomicron fractions (52) to prevent a too heavy contamination of serum proteins The present study demonstrated that layering was helpful in diminishing contamination of adjacent VLD-LP and serum proteins in the isolation of any VLD-LP subfraction Repeated ultracentrifugations, "washings" of the resuspended LP were always necessary, however These washings were performed at the same gravitational force as the preceding isolation step The goal of purification was to obtain a fraction that had the same protein composition after two consecutive washings and that was homogeneous on electrophoresis

Based on these experiences a standardized procedure was outlined for the isolation and purification of five arbitrarily chosen VLD-LP subfractions A, B, C, D, and E at centrifugal force $0\,1 \times 10^6$, $0\,6 \times 10^6$, $4\,8 \times 10^6$ $12\,6 \times 10^6$, and 139×10^6 g min, respectively As much as 45-50% of the original lipid content of a subfraction was lost by the washings Subjects with hyperglyceridemia, which had been characterized previously, were therefore used as donors to allow sufficient amounts of LP to be recovered and studied

In the chemical characterization of

micrographs (28), which also showed a spherical although somewhat flattened shape

The electrophoretic mobility of VLD-LP on agar gel (Figure 2) appear better explained in the light of the new knowledge of their protein moieties (Chapter V) The presence of β LP protein, and the known interference of β-LP with agar at different levels of protein concentration (54), may explain both the trailing of fraction C and the slight difference in mobility between fractions D and E (Figure 2)

It is apparent from the present study that LP of different size and chemical composition can be obtained by varying the speed and the duration of the ultracentrifugation Discrepancies in chemical and physical data in chylomicrons and VLD LP fractions obtained in different laboratories may be due to the lack of a standardized technique for the separation of these LP By the use of the experimentally obtained graph (Figure 5) relating Sf value of the LP maximum peak and ultracentrifugal force (g min) used for the isolation of the LP, such a standardization could be achieved

CHAPTER II

Partial delipidization (Papers II and III)

Complete delipidization of β-LP (55) and VLD LP (56) failed to give water soluble residues A nearly lipid free protein moiety was however obtained in water soluble form from α LP (57)

Methods for partial delipidization have thus far utilized a biphasic system of lipid solvents and water Using diethyl ether isolation of reproducible residues could not be obtained and there was a low yield of water soluble material particularly from VLD LP Since these were of primary interest in the present study a new method for partial delipidization was developed

Lyophilization of serum LP caused irreversible changes in the protein moiety (58, 59) However, it was known that the protein denaturation on freeze-drying may be prevented by the presence of glucose, glycerol, sucrose (58) and insoluble starch (60) This effect can possibly be explained by the hydroxyl groups of these compounds substituting for protein hydration water

Furthermore, the neutral lipid extraction was accelerated by the presence of starch, exposing the lyophilized LP to a large contact area with the lipid solvent (61) From ether delipidization experiments it was noted that the vulnerability of the LP protein moieties was due to the degree of phospholipid extraction as well as to the extent of dehydration By using a non polar lipid solvent, *n* heptane, less phospholipid was extracted and the yield of soluble phospholipid protein residues was improved

The lipid extraction was interrupted when all neutral lipids had been

repeated ultracentrifugations and resuspensions, besides removing contaminants, also cause some loss of cholesterol ester. This is the most likely explanation for the observation that the content of cholesterol ester was lower than expected in fractions D and E (Table B).

From the experimentally determined Sf° value and the estimated value for LP density, the approximate molecular weight (Figure B) of the average LP of the five subfractions was calculated. These molecular weights agree well with those obtained from the electron

Table B. *Lipid and protein composition of five VDL lipoprotein fractions and the β-lipoproteins. Data expressed as mole lipids per 100 amino residues*

Lipoprotein Fraction	LP Density (g/cc)	Mole Lipids per 100 Amino Acid Residues				
		FC	CE	PL	TG	PR
Fraction A	0.928	55	65	58	1060	100
Fraction B	0.936	49	73	55	590	100
Fraction C	0.957	22	37	33	170	100
Fraction D	0.977	13	26	23	84	100
Fraction E	0.989	11	20	16	61	100
β LP¹)	1.03	11	32	16	7	100

See Table A for abbreviations.
¹) Composition data from Oncley, 1963 (50).

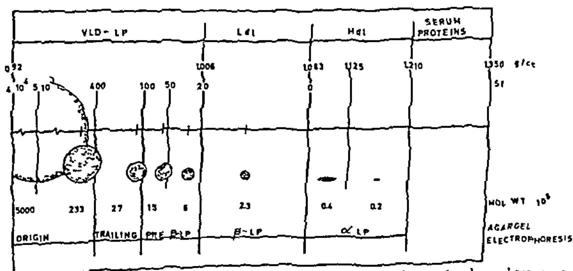


Figure B. The serum LP spectrum. Relationship between LP molecular weight, electrophoretic mobility on agar gel and the LP characteristics of hydrated density and Sf value. Data from the present study, except molecular weights of α LP (87) and β LP (50).

micrographs (28) which also showed a spherical although somewhat flattened shape

The electrophoretic mobility of VLD-LP on agar gel (Figure 2) appear better explained in the light of the new knowledge of their protein moieties (Chapter V) The presence of β LP protein, and the known interference of β LP with agar at different levels of protein concentration (54), may explain both the trailing of fraction C and the slight difference in mobility between fractions D and E (Figure 2)

It is apparent from the present study that LP of different size and chemical composition can be obtained by varying the speed and the duration of the ultracentrifugation Discrepancies in chemical and physical data in chylomicrons and VLD-LP fractions obtained in different laboratories may be due to the lack of a standardized technique for the separation of these LP By the use of the experimentally obtained graph (Figure 5) relating Sf value of the LP maximum peak and ultracentrifugal force (*g min*) used for the isolation of the LP, such a standardization could be achieved

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Lyophilization of serum LP caused irreversible changes in the protein moiety (58 59) However it was known that the protein denaturation on freeze-drying may be prevented by the presence of glucose, glycerol, sucrose (58) and in soluble starch (60) *This effect can* possibly be explained by the hydroxyl groups of these compounds substituting for protein hydration water

Furthermore, the neutral lipid extraction was accelerated by the presence of starch, exposing the lyophilized LP to a large contact area with the lipid solvent (61) From ether delipidization experiments it was noted that the vulnerability of the LP protein moieties was due to the degree of phospholipid extraction as well as to the extent of *dehydration* By using a non polar lipid solvent, n heptane less phospholipid was extracted and the yield of soluble phospholipid protein residues was improved

The lipid extraction was interrupted when all neutral lipids had been

removed This was determined by thin--layer chromatography of the consecutive lipid extracts (Figure 1) At the end of lipid extraction a certain amount of phospholipid, characteristic for each LP fraction, was left unextracted in the residue of all serum LP fractions (Table 1)

The composition of the phospholipid--protein residues suggests, in agreement with earlier studies (62, 63), that in all serum LP, the phospholipid component is bound to the protein moiety by stronger bonds than the neutral lipids, and also that the phospholipid in VLD and β-LP serves as a stabilizing agent *for the protein moiety*

The thin-layer chromatograms of the lipid extracts revealed that of the neutral lipids, the free cholesterol was more tightly bound in the β-LP than the cholesterol ester and triglyceride The evidence for a loosely bound cholesterol ester is in agreement with earlier findings (Paper I) of a cholesterol ester loss by repeated ultracentrifugations In α-LP apparently the lipid binding properties are different (64) In this LP, cholesterol ester is more tightly bound than free *cholesterol, as judged by the sequence* in the lipid removal

The carotenoids were studied by following the presence and intensity of their characteristic yellow color within LP and degradation products Their extractability showed an interesting similarity to that of the phospholipid, as might be expected from earlier studies *(65) Only little was extracted by* heptane and the major portion apparently remained with the phospholipid-protein residue VLD and β LP contained carotenoids, while α-LP and chylomicrons did not show any visible amount

Lipid moieties of the VLD-lipoproteins

CHAPTER III

VLD-lipoprotein composition and distribution in various hyperlipemic states

With the isolation of VLD LP subfractions in sera from various hyperglyceridemic donors it soon appeared that the distribution of total lipids in the five subfractions varied according to certain patterns In this chapter, a limited study on the total lipid (and LP) distribution among VLD-LP fractions in three clinical types of hyperlipemia, will be described

A brief review in the present knowledge of clinical typing of hyperlipemias will be given first

Fat induced (66), fat inducible (67) hyperlipemia or according to the recent typing of Fredrickson and Lees (67), *hyperlipemia type I*, is, in its severe familial form, quite rare It is characterized by the development of marked hyperlipemia, hyperglyceridemia even on a normal fat diet A low level of plasma post heparin lipolytic activity (PHLA) (67) and heparin unresponsiveness (68) is felt to indicate a low activity of LP lipase (in the tissue) (67) or a defective LP lipase system (68) Interestingly enough so far there have been no reports demonstrating an accelerated atherosclerosis among subjects with this lipid disorder, although serum triglyceride values often reach very high levels (Table C, subject H H)

Carbohydrate-induced (66), carbohydrate-inducible (67), dietary carbohydrate accentuated (69) hyperlipemia, *type IV* according to Fredrickson and Lees (67), has sensitivity of serum triglyceride level to dietary carbohydrate intake as a characteristic feature These subjects may also show other evidence of a defective glucose metabolism, abnormal intravenous glucose tolerance test (49, 66) abnormal tolbutamide test or a low serum insulin like activity (66) and often a family history of diabetes (49, 70) This type has a serum lipid pattern that has been found to indicate special proneness to coronary heart disease in young men (67)

Other types of primary hyperlipemia hyperglyceridemia occur In one other type suggested by Fredrickson and Lees (67), *the type V*, or *the fat and carbohydrate-inducible* form of hyperlipemia, the serum triglyceride levels were affected by both fat and carbohydrate intake Simplified this type may combine the features of type I and type IV

Clinical data and type of hyperlipemia of eight hyperglyceridemic subjects in-

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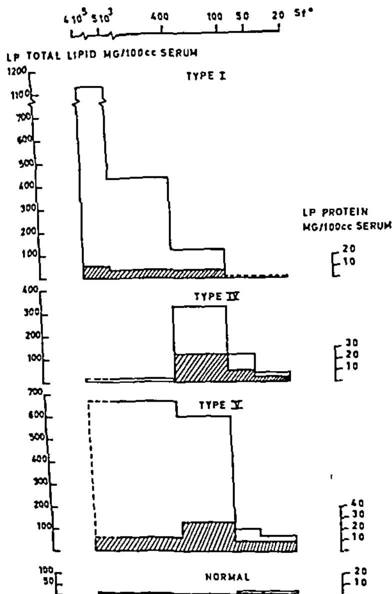


Figure C. Total lipid and LP protein distribution among VLD LP subfractions in three dietary types of hyperlipemia and in the normal subject. Distribution estimated from washed preparations, and expressed in mg per 100 cc serum.

cluded in this limited study are given in Table C These subjects were also used as donors in the work described in Paper I and, with the addition of pooled sera from five more subjects, in the investigations on the VLD-LP protein moieties, (Papers IV-V)

The subfractionation technique described in Paper I was utilized in this study Total lipid and, in washed prepa-

Table C *Clinical and laboratory data and type of hyperlipemia in eight hyperglyceridemic blood donors*

ct	Sex	Serum Lipids: Cholesterol (mg/100 cc)	Serum Lipids: Triglyceride (mg/100 cc)	Clinical History: Kinship	Clinical History: Alcohol Consumption	Clinical History: Evidence of Vascular Disease	Physical Signs: Xanthomas	Physical Signs: Hepatosplenomegaly	Tests: I v Heparin**)	Tests: I v Glucose Tolerance Test**)
	F	172	1645	Siblings	—	—	—	+	' Unresponsive	Normal
	F	140	685	Siblings	—	—	—	+	Unresponsive	Normal
	M	238	3488	Siblings	—	—	—	+	Unresponsive	Normal
	M	546	1131	Brothers	—	Intermittent claudication	Tuberous	+	Normal	Impaired
	M	314	398	Brothers	Moderate	—	Tuberous	+	Normal	?
	M	310	869	—	—	Myocard infarct at 47	—	—	Normal	Impaired
	F	470	797	—	Moderate	Myocard infarct at 54	—	—	Normal	Normal
	M	334	948	—	Excessive	Cerebral vasc. accident at 65	—	—	Normal	Mild diabetes

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me of these data the author is indebted to Drs O Brusco C W Robinson Jr and R P Howard

Table D *Per cent total lipid distribution among VLD lipoprotein fractions Comparison between unwashed and washed preparations*

Type of Hyperlipemia	Subject	Fraction Preparation	Per Cent Total Lipid Distribution: Sf 40 000	Sf 5 000	Sf 400	Sf 100	Sf 50	Sf 20
Type IV	O C	Unwashed	——— 16 2 ———			63 6	14 4	5 8
	O C	Washed	——— 10 1 ———			68 4	17 0	4 5

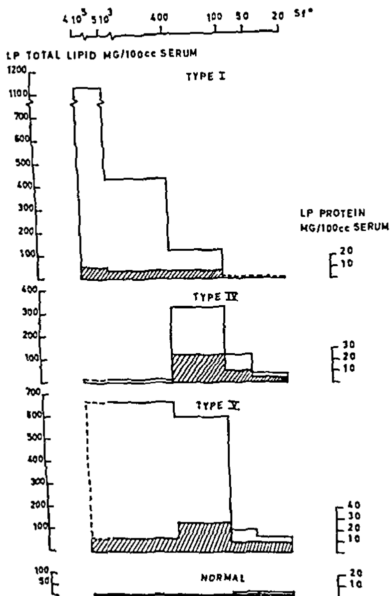


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The subfractionation technique described in Paper I was utilized in this study Total lipid and, in washed prepa-

Table C. *Clinical and laboratory data and type of hyperlipemia in eight hyperglyceridemic blood donors*

t	Serum Lipids		Clinical History			Physical Signs		Tests		T H lip
	Sex	Cholesterol (mg/100 cc)	Triglyceride (mg/100 cc)	Kinship	Alcohol Consumption	Evidence of Vascular Disease	Xanthomas	Hepatosplenomegaly	I v Heparin**)	I v Glucose Tolerance Test**)
	F	172	1645	Siblings	—	—	—	+	' Unresponsive	Normal
	F	140	685	Siblings	—	—	—	+	Unresponsive	Normal
	M	238	3488	Siblings	—	—	—	+	Unresponsive	Normal
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Type IV	O C.	Unwashed	———	16 2	———	63 6	14 4	5 8
	O C.	Washed	———	10 1	———	68 4	17 0	4 5

VLP LP distribution shown on analytical ultracentrifugation by Gofman et al (71)

In comparing composition data it seems that the VLD LP, Sf 20—100 in type IV showed a higher cholesterol ester content (Table G) As was stressed before (Chapter I) the cholesterol ester content is howeve the most variable of the components of any VLD LP fraction The results of Lindgren et al (28) indicated a similar difference in cholesterol ester in normo- and hyper glyceridemic sera

In two brothers (G T and R T) with clinical data suggesting type V hyper lipemia the total lipid distribution showed a combination of the patterns of type I and type IV Both VLD LP of Sf 100—400 and chylomicrons, Sf > 400 showed increased amounts of total lipid (Figure C Table E) This is in agreement with findings of Fredrick son and Lees from fat electrophoresis (67) Interestingly however the lipid particles recovered in Sf > 400 in type V in the present study showed a com position and appearance that differed from that of typical chylomicrons (Table H) Similarly Jobst and Schettler (72) observed a difference in composition between normal chylomicrons and those isolated from certain hyperlipemic sera

Table F Per cent total lipid distribution among VLD lipoprotein fractions Comparison between experiments at different oc[illegible] in subjects with hyperlipemia type I and type IV

Type of Hyperlipemia	Subject	Per Cent Total Lipid Distribution					
		Sf 40 000	Sf 5 000	Sf 400	Sf 100	Sf 50	Sf 20
Type I	H H	73 6	18 0	8 4	—	—	
	H H	67.2	25 4	7 4	—	—	
Type IV	O W	0 9	4 8	45 9	48 4	—	
	O W	—	3 0	65 6	23 4	8 0	

Table G Lipid and protein composition of VLD lipoproteins Sf 20—100 in hyperlipemic and normal subjects Data expressed as moles lipids per 100 amino acid residues

Lipoprotein Fraction			Mole lipids per 100 Amino Acid Residues				
Type of Hyperlipemia	Subject	Sf Range	FC	CE	PL	TG	PR
Type I postprandial	H H	Sf 20 — 400	16	5	31	175	100
Type IV postprandial	O W	Sf 20 — 100	14	29	23	61	100
Normal postprandial	A G	Sf 20 — 100	10	9	17	73	100
Normal alimentary	A.G	Sf 20 — 100	15	15	18	63	100

See Table A for abbreviations

rations, also total LP protein was quantitated The distribution was expressed either as the absolute amount of total lipid in mg per 100 cc of serum or as the per cent of the sum of total lipid in all VLD-LP subfractions

The per cent total lipid distribution was very similar whether the pattern was estimated on unwashed or washed preparations (Table D)

In fat-induced hyperlipemia, type I, (patient H H) the total lipid pattern showed a marked difference from that of the normal subject, with an absolute (Figure C) and relative maximum (Table E) in fractions A and B, the "chylomicron fractions" Identical distribution was seen in two siblings, S H and L F , with fat-induced hyperlipemia (data by Alaupovic and Furman, personal communication) This type of hyperlipemia satisfies the term hyperchylomicronemia suggested by Furman et al (69) The lipid particles of fraction A isolated from these subjects showed a lipid-protein composition similar to that of normal alimentary serum chylomicrons (Table J, Chapter IV) The increased fat particles in fat-induced hyperlipemia have been shown previously to have physical properties in common with normal chylomicrons (25, 66)

In subjects (O W , M A , and O C) with carbohydrate-induced hyperlipemia, type IV, a different total lipid pattern in the VLD-LP was observed The "peak" was within Sf 100—400, i e fraction C (Figure C, Table E) It should be stressed, however, that on different occasions in the same subject, variations occurred among the three major fractions i e fractions B, C and D, in this type of hyperlipemia (Table F) On the other hand, in type I very good reproducibility was the rule These variations in relative total lipid distribution seemed to reflect diet and food intake on the days preceding the test The present data in the normal subject and in subjects with hyperlipemia type IV, agreed with the

Table E *Per cent total lipid distribution among VDL lipoprotein fractions Comparison between that in three types of hyperlipemia and in the normal subject*

Type of Hyperlipemia	Subject	Per Cent Total Lipid Distribution					
		Sf 40 000	Sf 5 000	Sf 400	Sf 100	Sf 50	Sf 20
Type I	H H	67 2	25 4	7 4	—	—	
Type IV	O W	—— 3 0 ——		65 6	23 4	8 0	
	O C	—— 10 1 ——		68 4	17 0	4 5	
	M A	—— 17 9 ——		45 2	35 5	2 4	
Type V	R T	—— 19 6 ——		45 5	17.3	17 6	
	G T	—— 46 5 ——		41 3	7 2	5 0	
Normal	A G	10 7	11 8	18 7	58 8	—	

Table J Lipid and protein composition in human serum and chyle chylomicrons Data expressed as mole lipids per 100 amino acid residues

Source	Type of Hyperlipemia	Sf Range	Mole Lipids per 100 Amino Acid Residues				
			FC	CE	PL	TG	PR
Serum	Type I postprandial	Sf > 5 000	55	65	58	1060	100
	Type I postprandial	Sf 400 — 5 000	54	67	62	659	100
	Normal alimentary	Sf > 5 000	29	29	47	1760	100
	Normal alimentary[1])	~ Sf > 400	10	12	54	450	100
Chyle	"Cream [2])	~ Sf > 400	48	59	440	8250	100
	Corn oil [2])	~ Sf > 400	5	16	74	2170	100

See Table A for abbreviations

[1]) Composition data from Wood et al 1964 (142)

[2]) Thoracic duct chyle Composition data from Zilversmit 1965 (80)

hand lacked this protein moiety (76) The finding of β LP protein in a fraction, as in Sf 400—5000 of most hyperlipemic sera, would therefore suggest a contamination of VLD LP This was true for normal alimentary sera In studies by Scanu and Page (56) the presence of β LP protein was eliminated by purification of the chylomicron fraction

When different normal chylomicron fractions were compared the Sf > 5000 and the Sf 400—5000 of the hyperchylomicronemic sera agreed in composition with that of normal, alimentary serum chylomicrons, Sf > 5000 (Table J) When serum chylomicrons of the present study were compared with those isolated in other laboratories (Table J) the agreement in composition was good although differences in cholesterol content were observed Even qualitatively*) the phospholipids were similar in hyperchylomicronemic and normal, alimentary chylomicrons (Table K) In the same table are included, for comparison, the phospholipid distribution of other serum LP The chylomicrons appear to be closer to α than to β-LP

Chyle chylomicrons and serum chylomicrons differ as to composition A growing body of evidence points to drastic changes in the chyle chylomicrons when they enter the blood stream On in vitro incubation of dog chyle chylomicrons with serum the re isolated chylomicrons showed increase in free cholesterol and decrease in triglyceride and phospholipid (although a gain in lysolecithin was observed) (78) Human chyle chylomicrons also gain in protein content, from 0 2—0 6 % (76 79, 80) to 1 % (Paper I) Changes in electrophoretic mobility have also been observed (25, 81) Complete composition

*) The phosphatides were isolated and quantitated by the method of Nye Waterhouse and Marinetti (77)

Table H *Lipid and protein composition in VLD lipoproteins, Sf > 400 (chylomicron fractions) in various types of hyperlipemia Data expressed as mole lipids per 100 amino acid residues*

Lipoprotein Fraction			Mole Lipids per 100 Amino Acid Residues				
Type of Hyperlipemia	Subjects	Sf Range	FC	CE	PL	TG	PR
Type I postprandial	H H [1])	Sf 400 — 5 000	54	67	62	659	100
Type IV, postprandial	M A + O C [2])	Sf > 400	54	55	41	379	100
Type V postprandial	G T + R T [2])	Sf > 400	63	118	41	417	100

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[1]) Average of four experiments

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CHAPTER IV

The chylomicrons, characterization and composition in various states

Chylomicrons, the main vehicle in the exogenous fat transport, still lack an exact method for their isolation A variety of flotation procedures have been employed to separate them from the opalescent, triglyceride-rich VLD-LP (20)

Originally, Lindgren et al (73), suggested as chylomicrons in the normal, alimentary serum, a fraction floating at D 1 006 at a centrifugal force 0 1 × 10[6] g min Oncley (50) characterized the normal chylomicrons as LP of Sf 10,000 ± 5,000

The fatty acid composition (74) of the lipids of the fraction Sf > 400 (estimated by this author) from the normal, alimentary serum, showed good agreement with that of the ingested fats When LP of lower Sf value were included, the fatty acid discrepancies increased markedly (75)

In the present study, the presence in hyperchylomicronemia of large amounts of "particulate fat" (26) in fractions Sf > 5000 and Sf 400—5000, and the identical composition of these two fractions (Table J), indicated that in this lipid disorder (with low amounts of VLD-LP), Sf > 400 contained only chylomicrons

In other types of hyperlipemia the task of separating chylomicrons from VLD-LP was more difficult (20, 72) In type V, the Sf > 400 contained LP different in composition to both VLD-LP and chylomicrons (Table H)

Another way of characterization and identification of chylomicrons may be through their protein moiety The VLD-LP appear to be characterized by the presence of β-LP protein (Chapter V) Pure chyle chylomicrons, on the other

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Source	Type of Hyperlipemia	Sf Range	Mole Lipids per 100 Amino Acid Residues				
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CHAPTER IV

The chylomicrons, characterization and composition in various states

Chylomicrons, the main vehicle in the exogenous fat transport, still lack an exact method for their isolation A variety of flotation procedures have been employed to separate them from the opalescent, triglyceride-rich VLD-LP (20)

Originally, Lindgren et al (73), suggested as chylomicrons in the normal, alimentary serum, a fraction floating at D 1 006 at a centrifugal force $0\ 1 \times 10^{9}$ g min Oncley (50) characterized the normal chylomicrons as LP of Sf 10,000 ± 5,000

The fatty acid composition (74) of the lipids of the fraction Sf > 400 (estimated by this author) from the normal, alimentary serum, showed good agreement with that of the ingested fats When LP of lower Sf value were included, the fatty acid discrepancies increased markedly (75)

In the present study, the presence in hyperchylomicronemia of large amounts of "particulate fat" (26) in fractions Sf > 5000 and Sf 400—5000, and the identical composition of these two fractions (Table J), indicated that in this lipid disorder (with low amounts of VLD-LP), Sf > 400 contained only chylomicrons

In other types of hyperlipemia the task of separating chylomicrons from VLD-LP was more difficult (20, 72) In type V, the Sf > 400 contained LP different in composition to both VLD-LP and chylomicrons (Table H)

Another way of characterization and identification of chylomicrons may be through their protein moiety The VLD LP appear to be characterized by the presence of β-LP protein (Chapter V) Pure chyle chylomicrons, on the other

Table J *Lipid and protein composition in human serum and chyle chylomicrons Data expressed as mole lipids per 100 amino acid residues*

Source	Type of Hyperlipemia	Sf Range	Mole Lipids per 100 Amino Acid Residues				
			FC	CE	PL	TG	PR
Serum	Type I postprandial	Sf > 5 000	55	65	58	1060	100
	Type I postprandial	Sf 400 — 5 000	54	67	62	659	100
	Normal, alimentary	Sf > 5 000	29	29	47	1760	100
	Normal alimentary[1])	~ Sf > 400	10	12	54	450	100
Chyle	"Cream [2])	~ Sf > 400	48	59	440	8250	100
	"Corn oil" [2])	~ Sf > 400	5	16	74	2170	100

See Table A for abbreviations

[1]) Composition data from Wood et al 1964 (142)

[2]) Thoracic duct chyle Composition data from Zilversmit, 1965 (80)

hand lacked this protein moiety (76) The finding of β-LP protein in a fraction, as in Sf 400—5000 of most hyperlipemic sera, would therefore suggest a contamination of VLD LP This was true for normal alimentary sera In studies by Scanu and Page (56) the presence of β LP protein was eliminated by purification of the chylomicron fraction

When different normal chylomicron fractions were compared, the Sf > 5000 and the Sf 400—5000 of the hyperchylomicronemic sera agreed in composition with that of normal, alimentary serum chylomicrons Sf > 5000 (Table J) When *serum chylomicrons* of the present study were compared with those isolated in other laboratories (Table J) the agreement in composition was good although differences in cholesterol content were observed Even qualitatively*) the phospholipids were similar in hyperchylomicronemic and normal, alimentary chylomicrons (Table K) In the same table are included, for comparison, the phospholipid distribution of other serum LP The chylomicrons appear to be closer to α than to β LP

Chyle chylomicrons and serum chylomicrons differ as to composition A growing body of evidence points to drastic changes in the chyle *chylomicrons* when they enter the blood stream On *in vitro* incubation of dog chyle chylomicrons with serum, the re-isolated chylomicrons showed increase in free cholesterol and decrease in triglyceride and phospholipid (although a gain in lysolecithin was observed) (78) Human chyle *chylomicrons* also gain in protein content, from 0 2—0 6 % (76, 79, 80) to 1 % (Paper I) Changes in electrophoretic mobility have also been observed (25, 81) Complete composition

*) The phosphatides were isolated and quantitated by the method of Nye Waterhouse and Marinetti (77)

data of human thoracic duct chylomicrons has recently been reported in cream and corn oil feeding experiments (80) The differences in these chyle chylomicrons and the serum chylomicrons (Table J) correspond to the differences that could be expected from the *in vitro* studies

Table K *Phospholipid composition of serum lipoproteins*

otein Fraction		No of Samples	Per Cent of Total Lipid Phosphorus					Reference
			Lecithin	Sphingo-*myelin*	Lyso-*lecithin*	Phosphatidyl *Ethanolamine*	Unknown	
Chylomicrons[1])	Sf > 5 000	6	79	9 1	—	3 6	7 7	Present study
Chylomicrons[2])	Sf > 400	12	78 5	11 7	4 2	5 6	—	Wood et al 19(
.P	Sf 20 — 400	5	74 0	18 3	—	7 7	—	Nelson and Fre(1960 (143)
	Sf 0 — 20	5	*68 8*	*25 6*	—	5 6	—	Nelson and Fre(1960 (143)
	D 1 063 — 1 21	5	80 8	11 8	—	7 4	—	Nelson and Fre(1960 (143)
+ VHdl	D > 1 063	6	69 9	13 6	11 2	5 2	—	Phillips 1959 (1

ined from three siblings with type I hyperlipemia

ined from six normal subjects in the absorptive state

Protein moieties of the VLD-lipoproteins

CHAPTER V

Isolation and characterization of phospholipid-protein residues (Papers IV and V)

The method of lyophilization in the presence of starch, and lipid extraction with heptane, was utilized for the partial delipidization of serum LP (Chapter II) By this method neutral lipid free residues with characteristic phospholipid-protein ratios were obtained from any serum LP When characterized by analytical ultracentrifugation the water--soluble residue appeared as a schlieren pattern containing one or several of three sedimenting boundaries with approximate sedimentation coefficients 4S, 7S and 14S

Partially delipidized α LP, showed the 4S residue as a single homogeneous boundary (Figure 2A) The corrected sedimentation coefficient $S^{\circ}_{20,w}$ was 4 3S and the hydrated density 1 17 g/cc (Table I) After partial lipid extraction the β LP Sf 0—20, gave rise to a 14S residue This had a characteristic appearance (Figure 2B) and a tendency to aggregation causing minor faster moving products (20S, 27S) to appear The $S_{20,w}$ for the 14S residue was 14 5S (Table I) not taking into account the Johnston Ogston effect of the aggregate products

Partial delipidization of narrow and broad LP fractions of the VLD LP, Sf 20—400, gave evidence for three overlapping residues with sedimentation characteristics 4S, 7S and 14S This finding is in agreement with the suggestion of heterogeneity in VLD-LP (Paper I) Interestingly, after ether extraction of the VLD-LP a 4S and a 14S residue was identified in the analytical ultracentrifuge, but only inconstantly a 7S residue This is possibly due to the vulnerability of the 7S residue to the vigorous ether extraction procedure (Paper II)

The mixture of three residues in partially delipidized VLD LP prompted efforts toward their separation and isolation in sufficient amounts for complete lipid and protein characterization

Electrophoresis on various media, paper, cellulose acetate and starch gel (Figure I) showed that the three residues had a characteristic mobility, and that each was different from the others On starch block electropho esis the 4S the 7S and the 14S residues moved approximately, with α-1, α-2 and β-globulins, respectively Due to difficulties in the recovery of the phospholipid p otein material from starch powder (also experienced by Kunkel and Trautman) (82) a synthetic product, *Pevikon* C-870 (Stockholm Superfosfat AB S ockholm,

Sweden), was used for preparative purposes. In this medium, a powdered co-polymer of polyvinyl chloride and polyvinyl acetate, the residues showed the same electrophoretic mobility and even sharper separation due to less endosmosis (83). The recovery of water-soluble phospholipid-protein material from the *Pevikon* was 50—60 per cent protein by weight.

The electrophoresis on *Pevikon* block frequently allowed the isolation in relatively pure form of the 14S from the 4S and the 7S residues (Figure I). The 4S and the 7S residues, however, were not completely separated and were therefore eluted together and subsequently isolated by preparative ultracentrifugation.

The residues were characterized in the analytical ultracentrifuge by their values of $S^{\circ}_{20,w}$, hydrated density (Table I) and in immunoelectrophoresis and diffusion for their antigenic properties by specific anti-sera and by commercially available antibodies to human serum. The protein moiety, the apolipoprotein *), of each residue was (after denaturation) identified by its N-terminal amino acids and by its peptide patterns ("fingerprint") (Figures 4 and 5). The phospholipid portion of each residue was characterized by its phosphatide distribution (Table III). It could be concluded from these data that the 4S residue (and its protein moiety) isolated from the VLD-LP was identical to the 4S residue of the α-LP, containing apolipoprotein A. Furthermore, the 14S residue, and its protein moiety, obtained from VLD-LP was identical in physical and chemical characteristics with the 14S residue of the β-LP, containing apolipoprotein B (Tables I and II).

The 7S residue was characteristic of the VLD-LP. The $S^{\circ}_{20,w}$ found for the 7S residue was 6.9S and the hydrated density was 1.09 g/cc (Table I). Figure D shows the extrapolation of the inverted value of the sedimentation coefficients ($1/S_{20,w}$) to infinite dilution ($1/S^{\circ}_{20,w}$). The extrapolation of the hydrated density from the sedimentation coefficients at three solvent densities (as described in Paper V) is shown in Figure G (Chapter IX). Similar physical data were obtained for this residue when isolated from LP of Sf > 400 where it occurred singly, exclusively in sera of hyperlipemia type V. The phospholipid-protein ratio (weight by weight) of the 7S residue, 2.4, was higher than that for

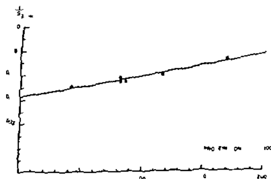


Figure D. The inverted value of the sedimentation coefficient ($1/S_{20,w}$) of the 7S residue at different protein concentrations (in mg per 100 cc). Extrapolation to zero concentration gives the inverted value of the sedimentation coefficient at infinite dilution ($1/S^{\circ}_{20,w}$).

*) The term apolipoprotein, as suggested by Oncley (144), designates the lipid free protein moiety of a lipoprotein. Thus apolipoprotein A designates the protein moiety of α lipoprotein, apolipoprotein B the protein moiety of β lipoprotein, and apolipoprotein C the protein moiety of a lipoprotein present in VLD lipoproteins (Sf > 20).

the 4S and the 14S residues, 0 8 and 0 7 respectively The high phospholipid content gave the 7S residue a large schlieren peak in the analytical ultracentrifuge (Figure 2C) Further heptane extraction of the LP fraction (*Sf* $>$ *400*) *containing* the 7S residue, did not reduce the phospholipid protein ratio, nor did it cause any apparent change in the physical characteristics of the residue The protein moiety of the 7S residue, the apolipoprotein C, gave one precipitin reaction with specific antisera, showed two N--terminal amino acids, serine and threo nine (Table II) and had peptide patterns different from those of the other two apolipoproteins (Figures 4 and 5)

The distribution of the residues among the VLD LP studied by analytical ultra centrifugation of partially delipidized subfractions, revealed that the 4S residue was present in chylomicrons (in fraction *Sf* $>$ *5000* of *hyperchylomicronemia* as a single component) and in the least dense VLD LP The 14S residue was characteristic for the heavier VLD LP at the right end of the VLD LP spectrum The 7S residue, finally, was found *within the intermediate chylomicron* and VLD LP fractions The distribution of the apolipoproteins according to Alaupovic, Ledford and Furman (to be published) is given in Figure E

The relative proportion of the three residues and indirectly the apolipoproteins was evaluated by protein determinations of the residues recovered from the block electrophoresis This method assumed an equal recovery of all three residues In type IV hyper-*lipemia the partially delipidized* VLD-LP (Sf$>$20), contained mainly apolipo protein B, 70 per cent, with about equal amounts of apolipoproteins A and C, 20 per cent and 10 per cent, respectively The type V hyperlipemia, was charac-

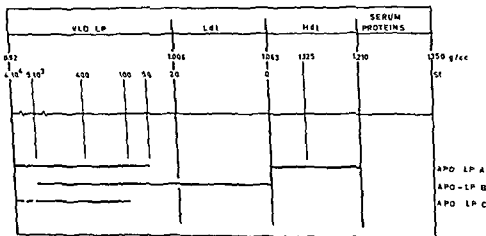


Figure E The serum LP *spectrum* *Distribution of the three LP protein* moieties, apolipoproteins A B and C among the serum LP fractions. For the VLD LP, determinations were done on hyper lipemic sera Data obtained from P Alaupovic J Ledford and R H Furman to be published

terized by the presence of more 7S residue, apolipoprotein C The relative proportions of the three apolipoproteins were 15 per cent, 50 per cent and 35 per cent for apolipoproteins A, B and C, respectively (Table IV) Normal sera showed evidence for the presence of all three apolipoproteins, but their relative proportions could not be studied because the amounts recovered were too little It could be expected, however, from the total lipid distribution with mainly VLD-LP, Sf 20—100, (Chapter III) that apolipoprotein B would be predominant in a fasting normal subject

No exact values for molecular weights of the lipid-free apolipoproteins could be obtained from the data However, some information on their size may be deduced from the relative proportion of the protein moiety in the molecular weight of the residue The experimentally determined molecular weights of the 4S and the 7S residues were 172,000 and 834,000 respectively (Table L) The Stoke minimum molecular weight (Paper I, p 598) assuming a spherical particle, calculated from $S^{o}_{20,w}$ and hydrated density, gave for the 14S residue a value of 1,040,000 These molecular weights of the residues were of the order of magnitude expected from thin-layer chromatography on Sephadex G-200 according to the method by Johansson and Rymo (84)

From these data the molecular size of the protein portion of the residues was estimated to be 100,000, 600,000 and 250,000 for apolipoproteins A, B, and C, respectively (Table L) In comparison, the direct determination of the molecular weight of the nearly lipid-free protein moiety of α-LP, the apolipoprotein A, has given values of 75,000 (57) and 36,000 (85), the latter being a monomer of the former

The β-LP protein, apolipoprotein B, has been found to contain two identical peptide chains of approximate molecular weight 380,000 each (86) The estimated value in the present study agreed fairly well with this data

Table L *Molecular weights of lipoproteins residues and apolipoproteins*

Lipoprotein Fraction		Residue						Apolipoprotein		
	Molecular Weight		Protein Content (%)	$S^0_{20\,w}$ (S)	Hydrated Density (g/cc)	Molecular Weight			Approximate Molecular Weight	
						Measured	Estimated[1])		Measured	Estimated
LP Hdl 2	375 000[2])	4 S	55	4 3	1 17	172 000	120 000	A	100 000	70 000
Hdl 3	175 000[2])									
P	2 300 000[3])	14-S	59	14 0	1 13	—	1 040 000	B	—	600 000
		7 S	30	6 9	1 09	834 000	600 000	C	250 000	180 000

Assuming a spherical particle For calculations see Paper I p 598
Lipoprotein molecular weight from Hazelwood 1958 (87)
Lipoprotein molecular weight from Oncley 1963 (50)

A value of 250,000 for the protein portion of the apolipoprotein C, would indicate, if the two peptide chains representing N terminal serine and threonine are of equal size a molecular weight of 125,000 (or fractions thereof) for each peptide The difference in estimated and experimentally determined molecular weights, 600 000 and 834,000 respectively, may indicate that the 7S residue has an ellipsoid rather than spherical shape

Discussion

CHAPTER VI

The structure of the VLD and the β-lipoproteins

Only limited information is available regarding the structure of serum LP Lipid extraction experiments in the present study (Chapter II) supplied certain suggestions as to the lipid-protein and lipid-lipid interactions in the LP On the basis of these findings a hypothetical LP model has been proposed Estimations were carried out assuming an ideal monomolecular distribution in the surface layer of the micellar particle

Of the four LP classes, the α-LP diverge most from the other serum LP, particularly in shape (87), and in lipid binding properties (Chapter II) (88) A growing body of evidence points to α-LP as a macromolecular compound (64, 89)

The β-LP, on the other hand, represent a micellar form of serum LP Estimations of the LP structure were based on this LP, assuming a spherical particle (90) Studies on the protein moieties of the serum LP, indicated the presence of triglyceride-rich β-LP within the heterogeneous VLD-LP Calculations were therefore also applied to the four VLD-LP subfractions Even data on chylomicrons, Sf > 5000, were included Chylomicrons have been established as true LP (Paper I) but contain α rather than β-LP protein (Chapter V)

In view of solubility properties, the LP show the characteristics of a protein This has been taken to indicate that the protein moiety *is present at or near the* LP surface and possibly makes up an outside shell of the LP structure (62, 89, 91) From the present data (Table M) it appears, however, that there is not enough protein in any VLD or β-LP to make a monomolecular layer, minimum thickness 7Å (92) around the entire LP molecule The surface area occupied by protein was estimated from the number of amino acid residues per LP molecule (Table A) and the value for the area per residue At an oil-water interface a film of 1 mg protein would occupy 0 7 m², or 13 6 Å² per amino acid residue (93) A value of 0 6 m² has also been reported (94)

With the protein interspersed with phospholipid, a larger area of the LP surface would be occupied The polar phospholipid, could also be expected to orient to the surface of the LP Lipid extraction experiments (Chapter II) postulated a strong bond between phospholipid and the protein moiety Furthermore, the phospholipid appeared to serve a function in stabilizing the protein

Table M *Lipoprotein total surface area compared with the area covered by available protein, protein phospholipid and protein phospholipid cholesterol in monomolecular films. Computed data are given for the average lipoprotein of five VLD lipoprotein fractions and the β-lipoproteins*

Lipoprotein Fraction			Lipid protein Area per g Mol Wt of LP		
	Diameter (Å)	Surface Area¹) (Å²)	PR²) (Å²)	PR + PL³) (Å²)	PR+FC+PL⁴) (Å²)
Fraction A	> 2572	> 2.1 × 10	0.6 × 10⁷	1.7 × 10⁷	2.6 × 10⁷
Fraction B	922	2.6 × 10⁶	0.5 × 10⁶	1.3 × 10⁶	1.9 × 10⁶
Fraction C	445	5.8 × 10⁵	1.7 × 10⁵	3.4 × 10⁵	4.2 × 10⁵
Fraction D	366	3.9 × 10⁵	1.6 × 10⁵	2.7 × 10⁵	3.2 × 10⁵
Fraction E	275	2.4 × 10⁵	0.9 × 10⁵	1.3 × 10⁵	1.5 × 10⁵
β-LP⁵)	192	1.0 × 10⁵	0.6 × 10⁵	0.9 × 10⁵	1.0 × 10⁵

See Table A for abbreviations

¹) LP surface area = $\pi (d-14)^2$ where d is LP diameter in Å

²) The value used for av. area per amino acid residue 13.6 Å² (0.7 m² per mg protein) was obtained at an oil water interface and 20 dynes per cm film pressure (93)

³) The value used for mean area per molecule of PL was 41.5 Å² (96)

⁴) The value used for mean area per molecule of FC and PL in a mixed film was 37 Å² (96)

⁵) Calculated from data of Oncley 1963 (50)

moiety (Chapter II) as well as the whole LP (95).

Calculations for the phospholipid area were done as for the protein with composition data taken from table A. For the average area per molecule in a film of lecithin a value of 41.5 Å (96) was used. The computations show that there is not enough of phospholipid and protein to cover the LP molecule with a monomolecular layer in any of the VLD or β LP (Table M).

Free cholesterol is known to form with phospholipid mixed films that are more compact than the pure phospholipid films. Phospholipid cholesterol associations occur commonly in living tissues particularly in cell membranes (97). Consecutive lipid extractions of β LP (Chapter II) revealed that free cholesterol was definitely harder to remove than the other neutral lipids, triglyceride and cholesterol ester. This finding was evidence in favour of a strong association between free cholesterol and the phospholipid protein complex or its constituents. The suggestion of free cholesterol and phospholipid appearing with the protein moiety at the LP surface seems to be supported by lipid exchange studies. Using labelled compounds phospholipid (98) and free cholesterol (99) exchanged freely among LP and corpuscular elements in the blood (100). This was not true for triglyceride and cholesterol ester.

On the basis of these indirect evidence it was assumed that free cholesterol and phospholipid form a mosaic in the film of protein. The surface area occupied by this association was estimated as above from composition data in table A. A value

of 37 Å^2 (96) was used for the area per molecule cholesterol and phospholipid in the mixed film (approximate molecular proportion 1 1) This combination of protein, phospholipid and free cholesterol (Table M) theoretically suffice to form a monomolecular distribution around the β-LP and possibly the chylomicrons In the latter, however, it should be stressed that the minimum LP surface area is given From these estimations the VLD-LP were suggested to contain a "loose" surface layer lacking the ideal monomolecular distribution due to large size, insufficient amounts of membrane constituents, or both The significance of this "loose" membrane in the stability and metabolism of VLD-LP is an intriguing question that will be further touched upon in connection with the LP-metabolism

Data in Table A, and the approximate molecular volume of tristearate, 1600 Å^3, and cholesteryl stearate, 1100 Å^3, allowed estimations of the total volume of the packed molecules of triglyceride and cholesterol ester, in the LP The values used for the molecular volumes were in agreement with those found in the crystalline state 1493 Å^3 and 1120 Å^3 for tristearate and cholesteryl stearate, respectively (S Abrahamsson and K Larsson, personal communication) It could be established that the space inside the surface membrane, with a diameter of d-14Å, was large enough for the packed molecules of cholesterol ester and triglyceride (Table N) Even enough additional space, about 10 % of the total volume, was left to allow a liquid distribution of these molecules It is apparent, however, that this "inner" space could

Table N *Lipoprotein total volume compared with the volume of packed molecules of available cholesterol ester and triglyceride Computed data are given for the average lipoprotein of five VLD lipoprotein fractions and the β lipoproteins*

Lipoprotein Fraction			Volume of Packed Lipid Molecules per g Mol Wt of LP		
	Diameter (Å)	Volume[1]) (Å^3)	CE[2]) (Å^3)	TG[3]) (Å^3)	CE + TG (Å^3)
Fraction A	> *2572*	> *8 7×10^9*	*0 3×10^9*	*8 0×10^9*	*8 3×10^9*
Fraction B	922	3 9×10^8	0 3×10^8	3 4×10^8	3 7×10^8
Fraction C	445	4 2×10^7	0 5×10^7	3 3×10^7	3 8×10^7
Fraction D	366	2 3×10^7	0 3×10^7	1 6×10^7	1 9×10^7
Fraction E	275	9 3×10^6	1 4×10^6	6 1×10^6	7 5×10^6
β LP[4])	192	2 9×10^6	1 4×10^6	0 5×10^6	1 9×10^6

See Table A for abbreviations

[1]) Volume $= \pi \frac{(d-14)^3}{6}$ where d is lipoprotein diameter in Å

[2]) The value used for molecular volume of cholesteryl stearate 1100 Å^3 was obtained from mol wt (654) density (0 99 g/cc) and the Avogadro number

[3]) The value used for molecular volume of tristearate 1600 Å^3 was obtained from vol wt (893) density (0 92 g/cc) and the Avogadro number

[4]) Calculated from data of Oncley 1963 (50)

not accommodate any large quantity of LP water, as suggested in earlier LP models (89) Recently the water content of β LP has been established as less than 10 % by weight (50) The water content of the VLD LP appears to be of the same order of magnitude, as judged from agreement in hydrated and anhydrous density (Paper I) This relatively low water content would mainly account for the hydration of the protein moiety In this connection the water molecules would be lodged with the protein moiety, possibly extending perpendicularly out from the LP surface (101)

Interestingly, Zilversmit (102), in studies on dog chyle chylomicrons found evidence for a similar LP structure The disrupted and isolated membranes appeared to contain protein, phospholipid, free cholesterol and a few per cent triglyceride The chylomicron membrane surrounded an oil phase', composed of cholesterol ester and triglyceride

Vandenheuvel (89) in 1962 suggested a β-LP model, that shows marked differences from the one now suggested His model was based on a 60 % (by weight) LP water content with the major portion lodged as a central water core A spherical protein shell of 7 Å thickness was estimated to surround and cover the LP molecule, assuming an area of 35—38 Å^2 per amino residue All lipids were accommodated between the protein layer and the water core, in a 22 Å wide annular space

CHAPTER VII

Chylomicron metabolism

By definition, chylomicrons originate in the intestines Ninety per cent of the neutral fat content of chyle chylomicrons comes from ingested fat (103) while the major portion of the phospholipid content is of endogenous source (103) This latter finding indicates that phospholipid are either synthesized in the intestinal wall or transported there from the serum As to the protein moiety the same two possibilities of origin, intestinal derivation (79 104, 105) and serum contribution (69) have been discussed

Assuming that the α LP protein (76) even in man is the major and characteristic protein moiety of the chyle chylomicrons, it can be estimated that a biosynthesis of this protein in the intestinal mucosa could fill the need also for α LP A rate of 100 g chylomicron fat influx per day and a 0 5 per cent protein content in chyle chylomicrons (Chapter IV) would give a gain of 0 5 g of α LP protein in the serum daily With a total amount of 3—4 g α LP protein in the serum and a half life of about 4 days (69) this would be enough to be the single source of α LP protein On the other hand, much evidence indicates a serum contribution of the serum chylomicron protein When I^{131}-labelled α-LP protein was injected into man, the

of 37 Å² (96) was used for the area per molecule cholesterol and phospholipid in the mixed film (approximate molecular proportion 1 1) This combination of protein, phospholipid and free cholesterol (Table M) theoretically suffice to form a monomolecular distribution around the β LP and possibly the chylomicrons In the latter, however, it should be stressed that the minimum LP surface area is given From these estimations the VLD LP were suggested to contain a "loose" surface layer lacking the ideal mono molecular distribution due to large size, insufficient amounts of membrane constituents, or both The significance of this "loose" membrane in the stability and metabolism of VLD LP is an intriguing question that will be further touched upon in connection with the LP metabolism

Data in Table A, and the approximate molecular volume of tristearate, 1600 Å³, and cholesteryl stearate, 1100 Å³, allowed estimations of the total volume of the packed molecules of triglyceride and cholesterol ester, in the LP The values used for the molecular volumes were in agreement with those found in the crystalline state 1493 Å³ and 1120 Å³ for tristearate and cholesteryl stearate, respectively (S Abrahamsson and K Larsson, personal communication) It could be established that the space inside the surface membrane, with a diameter of d-14Å, was large enough for the packed molecules of cholesterol ester and triglyceride (Table N) Even enough additional space, about 10 % of the total volume, was left to allow a liquid distribution of these molecules It is apparent, however, that this "inner" space could

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	Diameter (Å)	Volume[1] (Å³)	CE[2] (Å³)	TG[3] (Å³)	CE + TG (Å³)
Fraction A	> 2572	> 8 7×10⁹	0 3×10⁹	8 0×10⁹	8 3×10⁹
Fraction B	922	3 9×10⁸	0 3×10⁸	3 4×10⁸	3 7×10⁸
Fraction C	445	4 2×10⁷	0 5×10⁷	3 3×10⁷	3 8×10⁷
Fraction D	366	2 3×10⁷	0 3×10⁷	1 6×10⁷	1 9×10⁷
Fraction E	275	9 3×10⁶	1 4×10⁶	6 1×10⁶	7 5×10⁶
β LP[4]	192	2 9×10⁶	1 4×10⁶	0 5×10⁶	1 9×10⁶

See Table A for abbreviations

[1]) Volume $= \pi \frac{(d-14)^3}{6}$, where d is lipoprotein diameter in Å

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[3]) The value used for molecular volume of tristearate 1600 Å³ was obtained from mol wt (893) density (0 92 g cc) and the Avogadro number

[4]) Calculated from data of Oncley 1963 (50)

Table O *Hypothetical transformation of chylomicrons into α lipoproteins Composition of computed conversion steps compared with that of experimental fractions Data expressed as mole lipids per 100 amino acid residues*

Lipoprotein Fraction		LP Density	Mole Lipids per 100 Amino Acid Residues				
Hypothetical Transformation	Experimental Fraction	(g/cc)	FC	CE	PL	TG	PR
Chylomicrons	Fraction A	0 928	55	65	58	1050	100
TG Hydrolysis I		0 936	55	65	58	650	100
	Fraction B	0 936	49	73	55	590	100
Protein Dimerization I		0 941	28 (55)	33 (65)	29 (58)	325 (650)	100 (200)
TG Hydrolysis II		0 957	28 (55)	33 (65)	29 (58)	169 (337)	100 (200)
	Fraction C	0 957	22	37	33	170	100
Protein Dimerization II		0 979	14 (55)	16 (65)	15 (58)	84 (337)	100 (400)
	Fraction D	0 977	13	26	23	84	100
TG Hydrolysis III		1 083	14 (55)	16 (65)	15 (58)	3.3 (13)	100 (400)
	Hdl 2)	1 09	6	12	13	3.2	100

See Table A for abbreviations

') Compos tion data from Oncley and Gurd 1953 (145)

LP density (D_{LP}) as related to changes in mole lipid composition*)

This relationship in the case of cylomicron degradation was

$$D_{LP} = \frac{22\,6+42\,1+43\,6+0\,817X+0\,156Y}{21\,3+42\,5+45\,0+0\,893X+0\,117Y}$$

where X is the triglyceride and Y the amino acid residue content For Y a value of 100 200 and 400 was used for the original fraction and the two polymerization steps, respectively Protein dimerization steps were chosen at appropriate levels and the degree of triglyceride hydrolysis chosen to match the density of the experimental fractions The interesting agreement in composition of the estimated products and the experimental fractions is demonstrated in Table O Numbers in parentheses correspond to the data in Figure F in which the metabolism of chylomicrons into α LP is visualized (The dashed curve, represents a conversion by triglyceride removal alone) In the last hydrolysis step where the LP increase in density from 0 97 to 1 09 g/cc, only a relatively small amount of triglyceride (from 84 to 3 mole per 100 amino acid residues) have to be hydrolyzed as compared to the earlier steps Thus a rapid last metabolic step may explain the lack of chylomicron protein, α-LP protein within this density range However, other possi-

*) Molecular weights and values of density of lipid components and amino acid residues are given in Table A and Paper I page 598 respectively

chylomicron appearing in serum after fat-feeding contained a high portion of labelled α-LP protein (69) This would suggest that the newly synthesized chylomicrons contain protein also from the serum pool

The chylomicrons enter the blood stream by way of the thoracic duct In serum the chylomicrons change in chemical composition and physical characteristics, among other changes there is a gain in protein content and loss in triglyceride moiety (Chapter IV) Their metabolism is rapid In man, the serum chylomicrons have a half-life of 17 minutes (106) It is suggested from *in vivo* studies (28, 107, 108), that the *serum chylomicron* goes *through a* stepwise reduction in size and increase in density, through the loss of lipids, predominantly glycerides The glyceride moiety is hydrolyzed through the action *of LP lipase into glycerol and fatty* acids The fatty acids released are transported as a complex with albumin (109, 110) The appearance of mono- and *diglycerides (111, 112)* as well as fatty acids *(113)* in *serum during chylomicron* clearing support these concepts In regard to their protein moiety (Chapter V), it may well be assumed that chylomicrons metabolize into α-LP During chylomicron clearing in man *(107, 114)*, as well as in the dog (115), an increase in α-LP lipid has been established However the absence of α-LP or α-LP protein within the β-LP region, D 1 006—1 63, has been used as an argument against a successive metabolism of chylomicrons into *α-LP*

In the present study, the molar composition data (Table B) in fractions containing α-LP protein, i e fractions A through D, show that if an interconversion among these LP occurs this cannot be caused by triglyceride removal *alone but by loss of cholesterol* and phospholipid, as well The fate of cholesterol and phospholipid moiety released under such circumstances is hard to explain with our present knowledge of LP Thus other interpretations have to be searched for The difference in protein and triglyceride content at the conversion of the chyle chylomicrons into serum chylomicrons (Chapter IV), suggested the possibility of a protein uptake or polymerization in addition to triglyceride removal Alpha-LP and its protein moiety possess good qualifications for this function, because they have a pronounced tendency to polymerization (85, 116) a high affinity for fat emulsions *(56) and the specific ability* to activate substrates for LP lipase (56, 117)

Composition and density of chylomicron degradation products obtained by *protein uptake* and successive *triglyceride removal, was estimated* and compared (Table O) with the actual data of experimental fractions B through D (in which α-LP protein was present)

The equation,

$$D_{FC} \times m_1 \times MW_{FC} + D_{CE} \times m_2 \times MW_{CF} + D_{PL} \times m_3 \times MW_{PL} + D_{TG} \times m_4 \times MW_{TG} + D_{PR} \times m_5 \times MW_{PR} = D_{LP} \times (m_1 \times MW_{FC} + m_2 \times MW_{CE} + m_3 \times MW_{PL} + m_4 \times MW_{TG} + m_5 \times MW_{PR})$$

where D stands for density, m for molar concentration and MW for molecular weight, was used for the calculation of

CHAPTER VIII

VLD lipoprotein metabolism

VLD LP, Sf 50—100 (121), and Sf > 12 (122) are biosynthesized by the liver. Support for this is given by studies in animals with liver slice techniques (121, 123) and liver perfusion (121, 122, 124). Beta LP protein apolipoprotein B was identified by immunochemistry (121) in the newly synthesized VLD LP. (Compare discussion on identification of protein moieties in Chapter IX). The rate of this LP synthesis is possibly related to factors that control triglyceride biosynthesis, such as fatty acid mobilization from adipose tissue (125, 126) and *de novo* synthesis of fatty acids from carbohydrate (66, 127, 128). The excess of fatty acids is incorporated into triglycerides in the liver (129) and by way of liver triglycerides (130) secreted into the blood stream. The increased serum triglycerides are transported with VLD LP (131) opalescent β particles (128). In the present study, VLD LP with β LP protein possibly of hepatic origin, were found within Sf 20—5000 in hyperlipemic states (Chapter V).

A conversion of VLD LP from higher into lower flotation rates and ultimately into β LP, through the removal of glycerides, has been suggested from experiments *in vivo* with I^{131} labeled VLD LP (132) and *in vitro* by the lipolytic action of LP lipase in post heparin plasma (133, 134). A metabolism of VLD LP into β LP seems likely from point of view of LP protein distribution (Figure E). Furthermore the mole composition data particularly of fractions D and E as compared to that of β LP suggest a metabolic relationship between these fractions (Table B). Composition and density of intermediate metabolic products were estimated as for the

Table F. *Hypothetical transformation of VLD lipoprotein into β-lipoproteins. Composition of computed conversion steps compared with that of experimental fractions. Data expressed as mole lipids per 100 amino acid residues*

Lipoprotein Fraction		LI Density (g/cc)	Mole Lipids per 100 Amino Acid Residues				
Hypothetical Transformation	Experimental Fraction		FC	CE	PL	TG	PR
TG Addition II		0.977	11	31	16	83	100
	Fraction D	0.977	13	26	23	84	100
TG Addition I		0.989	11	31	16	61	100
	Fraction E	0.989	11	20	16	61	100
β-LP)		1.03	11	31	16	7	100

See Table A for abbreviations

) Composition data from Oncley (50)

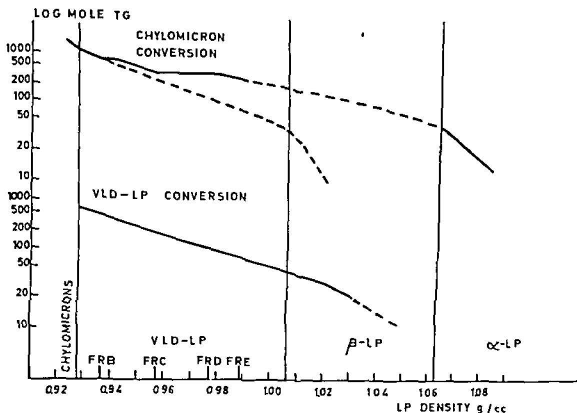


Figure F Hypothetical transformation of chylomicrons and VLD LP into LP of higher density Change in LP density as related to loss of triglyceride expressed as log mole triglyceride (TG) per 100 amino acid residues Chylomicron conversion by triglyceride loss alone (dashed curve) and by triglyceride loss and two steps of dimerization (doubling of the protein content) (solid curve) VLD LP conversion by triglyceride loss alone Solid line in the chylomicron conversion (upper curve) indicates fractions containing apolipoprotein A, and in the VLD LP conversion the presence of apolipoprotein B For numerical data see also Tables O and P

ble explanations for the "β gap", the lack of α-LP protein within the β-LP region, have to be kept in mind An extravascular metabolism (118, 119) of the chylomicron degradation products with the concomitant release of α-LP or α-LP protein back into the serum must be considered

The protein moiety, added to the chylomicron at the successive conversions, would most likely represent α-LP protein in a process of polymerization In serum, α-LP protein occurs as a lysolecithin complex (120) in the very-high-density lipoproteins (VHdl) (33, 34) This fraction could be a source of the α-LP protein However, the presence of phospholipid with the added protein in the chylomicron conversion is not suggested from composition data (Table O) The apolipoprotein C may possibly, under certain circumstances, be an alternate protein moiety, serving in chylomicron metabolism in the form of a phospholipid-protein complex (Chapter IX)

CHAPTER VIII

VLD-lipoprotein metabolism

VLD LP, Sf 50—100 (121) and Sf > 12 (122) are biosynthesized by the liver Support for this is given by studies in animals with liver slice techniques (121, 123) and liver perfusion (121 122, 124) Beta LP protein, apolipoprotein B was identified by immunochemistry (121) in the newly synthesized VLD LP (Compare discussion on identification of protein moieties in Chapter IX) The rate of this LP synthesis is possibly related to factors that control triglyceride biosynthesis such as fatty acid mobilization from adipose tissue (125, 126) and *de novo* synthesis of fatty acids from carbohydrate (66, 127, 128) The excess of fatty acids is incorporated into triglycerides in the liver (129) and by way of liver triglycerides (130) secreted into the blood stream The increased serum triglycerides are transported with 'VLD LP (131) opalescent β particles (128) In the present study, VLD LP with β LP protein possibly of hepatic origin, were found within Sf 20—5000 in hyperlipemic states (Chapter V)

A conversion of VLD-LP from higher into lower flotation rates and ultimately into β-LP, through the removal of glycerides, has been suggested from experiments *in vivo* with I^{131} labeled VLD LP (132) and *in vitro* by the lipolytic action of LP lipase in post heparin plasma (133, 134) A metabolism of VLD LP into β LP seems likely from point of view of LP protein distribution (Figure E) Furthermore the mole composition data particularly of fractions D and E as compared to that of β-LP suggest a metabolic relationship between these fractions (Table B) Composition and density of intermediate metabolic products were estimated as for the

Table P *Hypothetical transformation of VLD lipoproteins into β-lipoproteins Composition of computed conversion steps compared with that of experimental fractions Data expressed as mole lipids per 100 amino acid residues*

Lipoprotein Fraction		LP Density (g/cc)	Mole Lipids per 100 Amino Acid Residues				
Hypothetical Transformation	Experimental Fraction		FC	CE	PL	TG	PR
TG Addition II		0 977	11	31	16	83	100
	Fraction D	0 977	13	26	23	84	100
TC Addition I		0 989	11	31	16	61	100
	Fraction E	0 989	11	20	16	61	100
β-LP¹)		1 03	11	31	16	7	100

See Table A for abbreviations

¹) Composition data from Oncley (50)

chylomicron α-LP conversion (Chapter VII), although only triglyceride removal was taken into consideration

Calculations were based on data of the end product, the β-LP, to which triglyceride was added The equation relating change in LP density (D_{LP}) with change in triglyceride was,

$$D_{LP} = \frac{4\ 7+20\ 4+11\ 9+0\ 817X+15\ 5}{4\ 4+20\ 5+12\ 2+0\ 893X+11\ 7}$$

where X is the triglyceride content This equation is satisfied by the curve in Figure F The composition of the estimated conversion products agree well with those of the experimental fractions, *Table P Regarding differences in chol*esterol ester, see also Chapter I

No calculations on Sf 100—5000 were made although the presence of β-LP protein (Figure E) would indicate the presence of VLD-LP in these fraction The occurence of these LP in hyperlipemic states only (Chapter III) may indicate that they are an abnormal form of LP, not properly metabolized An imbalance in lipids and protein, through an excess of triglyceride (from fatty acid mobilization) and insufficient protein supply (by liver synthesis) at the LP formation, may give rise to these triglyceride-loaded VLD-LP, Sf 100—5000 These LP cannot be converted by triglyceride removal alone, according to the assumption in Chapter VII, but need protein addition or polymerization The vulnerability of the β-LP protein (Paper II), *however, speaks against the possi*bility of this protein moiety occuring in a lipid-free or lipid-poor form able to serve a polymerizing function

The fact that both apolipoprotein A and C are found within these fractions, *Sf 100—5000, makes it a possibility that* any of them may participate in the VLD-LP conversion The evidence for apolipoprotein A as an active factor in the metabolism of triglyceride-rich LP was emphasized in Chapter VII The qualifications for apolipoprotein C to participate in LP metabolism will be discussed in the next chapter

CHAPTER IX

The third protein moiety, apolipoprotein C

In the present study on serum chylomicrons and VLD-LP, the protein moieties have been the major interest Electrophoresis of lipid-rich LP (Figure 2, Paper I) did not necessarily reveal the true mobility of the protein moieties, but showed a pattern obscured by the influence of lipids

Immunoelectrophoresis, utilizing specific anti-LP sera, showed the presence in VLD-LP of only one protein moiety, the β-LP protein (55, 135, 136)

Not until experiments on the degradation of chylomicrons and VLD-LP (56, 137, 138) were performed, was evidence for a second, "hidden", protein moiety disclosed Alpha-LP protein was identified by immunochemical studies in ether extracted chylomicrons (56) and recently also in VLD-LP (138)

In disagreement with these results, studies by N terminal amino acid analysis indicated the presence of as many as four different protein moieties Thus in addition to N terminal aspartic — and glutamic acid contributed by the α and β LP proteins, respectively, N terminal serine and threonine were also detected (86 134, 139, 140)

In the present study, a protein moiety contributing N terminal serine and threonine was isolated for the first time*) Recovered after mild delipidization of VLD LP or chylomicrons, the third protein moiety could be visualized in the analytical ultracentrifuge as a sedimenting phospholipid containing residue, the 7S residue (Chapter V) By using specific antibodies to VLD LP and to whole serum, one characteristic precipitin reaction was obtained in immunoelectrophoresis and diffusion (Chapter V) In view of the fact that the 7S residue (and its protein moiety, the apolipoprotein C) has not been described before some additional evidence for its actual existence will be taken up

a) Randomly adsorbed protein impurities would be eliminated by the purification in the isolation procedure (Chapter I) The high phospholipid content of the 7S residue furthermore excluded the possibility of a *serum protein contaminant*

b) The 4S and the 14S residues, representing previously recognized protein moieties, α and β LP proteins respectively, may theoretically give rise to products of any physical characteristics Differences in size, the 7S being the largest (Chapter V), apparently exclude the possibility of this residue being a *degradation product* of the 4S and the 14S residues The 7S residue was also studied for the presence of *polymerization products* by the addition of urea and an anionic surfactant (sodium dodecyl sulfate) Urea in concentrations between 1 and 4 M or the surfactant agent in a concentration of 0 10%, produced no demonstrable alterations in ultracentrifugal characteristics as it would if the 7S residue had been a polymer

c) Prolonged and repeated lipid extractions with heptane were carried out on fractions containing the 7S residue (Chapter V) These experiments excluded the possibility of the 7S residue being the results of a *defect in the delipidization procedure*

In order to visualize the differences (and similarities) in the physical characteristics between the 7S, 4S and 14S residues a graph was constructed (Figure G) showing a plot of sedimentation coefficient ($S \times \eta$) *versus* hydrated density (ϱ) in the same manner as used in the extrapolation of hydrated density (see Paper V) The similarity in sedimentation coefficients of the 7S residue, the Hdl-2 and the 4S residue within certain solvent densities 1 03—1 07 g/cc, may explain the inability to detect the 7S residue *in* the analytical ultracentrifuge in earlier experiments with degraded VLD-LP The slopes of the chylomicrons, Sf > 5000, the VLD LP fraction E Sf 30,

*) The question as to whether the presence of two N terminal amino acids indicated two *different* proteins or two peptide chains linked together cannot be fully settled at *this time* However up until now the immunological studies have shown evidence for only one antigenic protein moiety (Chapter V)

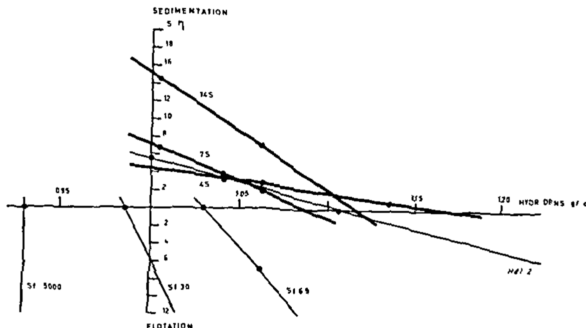


Figure G Sedimentation coefficient (S x η) and flotation rate (Sf x η) *versus* solution density (ρ) of the three residues the 4S, the 7S and the 14S, and of four LP fractions the chylomicrons (Sf > 5000), the VLD LP fraction E (Sf 30), the β LP (Sf 6 9) and the α LP (Hdl 2) The point at which each line meets the abscissa gives the respective hydrated densities of the residues and the LP Data from the present study, except for Sf 6 9 (50) and Hdl 2 (87)

and the β-LP, Sf 6 9, are also given in this figure for comparison

The probability of apolipoprotein C being present as a phospholipid complex in the serum, i e in a native apolipoprotein form outside the VLD-LP, has not been possible to confirm in the present study Some narrow, not completely characterized LP fractions with an electrophoretic mobility of α-2 (similar to that of the 7S residue) have previously been described by Shore and Shore (134) in Sf 0-20, by Lewis et al (141) in D 1 0631-1 10 and by Oncley (50) in D 1 05 Investigations are now under way to test these fractions for the presence of apolipoprotein C

Two conclusions from the hypothetical estimations on the chylomicron and VLD-LP structure (Chapter VI) and metabolism (Chapters VII and VIII) gave some suggestions for a possible function for the apolipoprotein C or its phospholipid-protein complex

Computations of the LP structure suggested that VLD LP, in contrast to β-LP, lacked enough protein-phospholipid-free cholesterol material for an ideal monomolecular distribution in the LP membrane A "loose" membrane was suggested for these LP, leading possibly to a structural instability, with tendency for disruption and loss of neutral lipids, particularly cholesterol ester and triglyceride (Chapter I) The apolipoprotein C, with a high phospholipid-binding capacity may, in the form of a phospholipid-protein complex, be equiped to serve as a stabilizer for these LP

Secondly, in the hypothetical conversion of chylomicrons and VLD LP into LP of higher density, the chylomicrons appeared to have sufficient amounts of polymerizing protein in all situations, except possibly in a-α lipoproteinemia On the other hand, when applied to the triglyceride loaded VLD LP of Sf>100 the conversion theory does not fit, due to a definite lack of circulating apolipoprotein B The apolipoprotein C, being stable in phospholipid-rich complexes, and possibly present as such a complex in the serum may be able to serve as a substitute for the apolipoprotein B in the metabolism of these VLD LP

Only limited informations is available at present on this third LP protein moiety and its phospholipid-protein complex It is speculated, however, that it may participate with α and β LP in the structure and metabolism of the most triglyceride rich VLD LP and possibly the chylomicrons It should be stressed at this point, however, that no other metabolic system in the body is known to be supplied with such a substituting factor

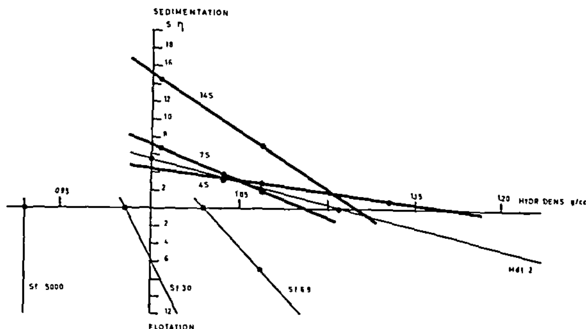


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characteristic phospholipid protein residues with approximate sedimentation coefficients 4S, 14S and 7S Partially delipidized VLD-LP, Sf>20 contained all three residues The distribution of the three residues among chylomicrons and VLD LP showed that the chylomicrons contained mainly the 4S residue and that the 14S residue was present in VLD LP The 7S residue was found within chylomicrons as well as VLD-LP

The three residues were separated and each characterized by its protein moiety The 4S contained α-LP protein apolipoprotein A the 14S contained the β LP protein, apolipoprotein B, and the 7S, finally, appeared to contain a third protein moiety not previously isolated, apolipoprotein C This latter protein gave as characteristic N terminal amino acids serine and threonine showed one precipitin band with antibodies to human serum and human serum VLD LP, and had peptide patterns different from those of the other two LP proteins

Chapter VI A possible model of the micellar LP, is discussed Physical characterization and chemical composition of the β LP are consistent with a mono molecular membrane containing an association of free cholesterol phospho lipid and protein surrounding a lipid core composed of cholesterol ester and triglyceride The composition of the VLD LP was insufficient for an ideal monomolecular layer covering their entire LP surface A loose membrane of similar appearance was suggested for these LP This loose membrane could explain the experimentally observed instability of the VLD-LP structure It was also estimated that enough space was available to accommodate for the cholesterol ester and triglyceride molecules (in liquid distribution) within the central sphere of all VLD and β-LP

Chapter VII A hypothetical intravascular metabolism of chylomicrons by their conversion into VLD LP and ultimately into α-LP was computed from the LP data This conversion assumed, besides triglyceride removal, also protein addition, possibly as a polymerization of α LP protein The computations gave intermediate conversion products with a composition remarkably similar to that of the experimental fractions

Chapter VIII The metabolism of VLD-LP by their conversion into β-LP was supported by estimations of the mole lipid composition of VLD and β-LP Conversion by intravascular *hydrolysis* of triglyceride appear applicable to the normally occuring VLD LP, Sf 20—100 On the other hand in less dense VLD-LP, Sf>100 present in excess in hyperlipemic states the metabolism may go through a different mechanism as judged from these estimations and known properties of the β LP protein

Chapter IX The third LP protein, apolipoprotein C, or its phospholipid protein complex, may serve a function in the transformation into LP of higher density of these less dense VLD-LP, Sf >100 and possibly under certain conditions also of the chylomicrons

Summary

Chapter I. A standardized procedure for the ultracentrifugal separation and purification of chylomicron and VLD-LP fractions was developed. With this method any number of narrow subfractions of these LP can be isolated. Five arbitrarily chosen fractions were isolated and characterized. Chemical and physical data of these LP indicated that the chylomicrons and the VLD-LP contained a continuous spectrum of heterogeneous LP of decreasing size and increasing density. Judged by the greater loss of certain lipids by the isolation-purification procedure, particularly cholesterol ester, a certain instability of their structure was suggested.

Chapter II. The protein moieties of the chylomicrons and the VLD-LP were studied to verify whether differences in these might be the cause of the LP heterogeneity. Lipid-free protein moieties of the chylomicrons and the VLD-LP could not be recovered in water-soluble form. A method for partial delipidization, particularly applicable to the lipid-rich VLD-LP, was developed. Repeated heptane extractions of LP that were lyophilized in the presence of insoluble starch gave reproducible, neutral lipid-free phospholipid-protein residues of all serum LP.

Certain differences in the extractibility of the lipids were observed.

Chapter III. Three different dietary types of hyperlipemia were each characterized by the presence in excess of certain LP fractions. Subjects presenting evidence for fat-induced hyperlipemia, (type I) (67), carbohydrate-induced hyperlipemia, (type IV), and a mixed dietary form, (type V), were studied in a limited number of cases and compared with the VLD-LP distribution in the normal subject. The type I hyperlipemia was characterized by large amounts of chylomicrons, the type IV of cholesterol and triglyceride-rich VLD-LP, Sf 100—400, and finally type V, showed the presence in excess of "atypical chylomicrons" as well as VLD-LP, Sf 100—400.

Chapter IV. Chylomicrons were difficult to separate and characterize, particularly in the presence of excessive amounts of VLD-LP as in most types of hyperlipemia. It is suggested that their characterization by protein moiety may be useful.

Chyle chylomicrons and serum chylomicrons showed certain differences in lipid-protein composition. These differences were taken into consideration in the discussion of their metabolism (Chapter VII).

Chapter V. The method of partial delipidization of the serum LP gave three

great share in this work Without her support and encouragement this thesis would never have been completed

This study was aided by grants from the U S Public Health Service (HTS 5403, HE 6221 and HE-2528), the American Heart Association, the Oklahoma Heart Association, the Swedish National Association for the Study of Heart and Lung Disease and the Swedish Oleomargarine Manufacturer Association for Nutrition Research

Acknowledgements

This work was started in 1960, and mainly completed at the Oklahoma Medical Research Foundation, Oklahoma City, USA, where I had the privilege of being a member of the group under the stimulating leadership of R H Furman, M D , Associate Professor of Research Medicine, University of Oklahoma School of Medicine, and Head of the Cardiovascular Section, Oklahoma Medical Research Foundation Dr Furman supplied me with excellent working conditions, the best guidance and a great deal of encouragement For more than three years, P Alaupovic, Ph D , Associate Professor of Research Chemistry, Department of Biochemistry, University of Oklahoma School of Medicine, and Assistant Head of the Cardiovascular Section, Oklahoma Medical Research Foundation, was my inspiring teacher and guide in the fundamentals of biochemical research I owe both of them my sincere thanks

Throughout this study, Professor L Werko, M D , Professor of Internal Medicine, Head of Department of Medicine I, University of Goteborg, has supported me in every way My introduction to the research on serum lipids and lipoproteins, I owe to B Hood, M D , Associate Professor, Department of Medicine I, University of Goteborg Professor Werko and Dr Hood initiated the exchange program in which I had the privilege of being the first participant G Welin, M D , Assistant Professor and Head of Department of Medicine, County Hospital, Molndal supported me by his never-ceasing enthusiasm and active interest in my work Professor O Mellander, M D , Professor of Medical Biochemistry, Head of Department of Medical Biochemistry, University of Goteborg, supplied me with laboratory facilities I express my sincere gratitude to them

I thank, the County Council of Goteborgs and Bohus lan for allowing me to combine my appointment at the County Hospital, Molndal with stay in Oklahoma

In Oklahoma City, Drs R P Howard, M D , and R H Bradford, M D , Ph D , and in Goteborg Drs K Cramér, M D , P Bjorntorp, M D , and B Johansson, M D , supplied me with advice and criticism, for which I am very grateful Mrs S Walrawen, Mr M Sullivan, Mr G Asher and Miss A W Fryer gave me excellent technical assistance and for secreterial aid I owe thanks to Mrs M Farmer, Oklahoma City, and Mrs K Nordén, Goteborg For skilful help in the preparation of graphs and figures, I thank Miss M McDearmon, Oklahoma City, and Mrs F Widlund, Goteborg

Finally, my wife, Agneta, has had a

50 ONCLEY J L. In Brain Lipids and Lipoproteins and the Leucodystrophies" Ed by J Folch Pi and H Bauer Elsevier Amsterdam 1963 p 1
51 LINDGREN F T NICHOLS A V UPHAM F T and WILLS R D J Phys Chem 66 2007 1962
52. BRAGDON J H HAVEL, R J and BOYLE, E J Lab Clin Med 48 36 1956
53 LOWRY O H ROSEBROUGH N J FARR A L. and RANDALL, R J J Biol Chem 193 265 1951
54 WALTON K W Immunochemistry 1 279 1964
55 SCANU A LEWIS L A and PAGE I H J Exp Med 108 185 1958
56 SCANU A and PAGE I H J Exp Med 109 239 1959
57 SCANU A LEWIS L A and BUMPUS F M Arch. Biochem 74 390 1958
58 LOVELOCK J E Proc Roy Soc. Ser B 147 427 1957
59 ONCLEY J L. WALTON K W and CORNWELL D G J Amer Chem Soc. 79 4666 1957
60 ANFINSEN C B and QUIGLEY T W Jr Circulation 8 435 1953
61 ZILVERSMIT D B J Lipid Res 5 300 1964
62. ONCLEY J L. GURD F R N and MELIN M J Amer Chem Soc. 72 458 1950
63 AVIGAN J J Biol Chem 226 957 1957
64 COOK W H and MARTIN W G Canad J Biochem. 40 1273 1962.
65 DZIALOSZYNSKI L. M MYSTKOWSKI E M and STEWART C. P Biochem J 39 63 1945
66 AHRENS E H Jr HIRSCH J OETTE, K FARQUHAR J W and STEIN Y Trans Ass Amer Physicians 74 134 1961
67 FREDRICKSON D S and LEES R. S Circulation 31 321 1965
68 FURMAN R H and ROBINSON C. W Jr Trans Amer Clin Climat. Ass 73 30 1961
69 FURMAN R H SANBAR S S ALAUPOVIC P BRADFORD R and HOWARD R. P J Lab Clin Med. 63 193 1964
70 ADLERSBERG D and WANG C. Diabetes 4 210 1955
71 GORMAN J W et al Plasma (Milan) 2 413 1954
72 FORST H and SCHETTLER G In "Internat. conf biochem pr bl lipids 3 (Brussels 1956) p 136
73 LINDGREN F T ELLIOTT H A and GOFMAN J W J Phys l. Coll d Chem 55 80 1951
74 KAYDEN H J KARMEN A and DUMONT A J Clin Invest 42 13 3 1963
75 DOLE V P JAMES A T WEBB J P W RIZACK M A and STURMAN M F J Clin Invest 38 1544 1959
76 RODBELL, M and FREDRICKSON D S J Biol Chem 234 562 1959
77 NYE W H R. WATERHOUSE, C. and MARINETTI G V J Clin Invest 40 1194 1961
78 MINARI O and ZILVERSMIT D B J Lipid Res 4 424 1963
79 BRAGDON J H J Lab Clin Med 52 564 1958
80 ZILVERSMIT D B J Clin Invest. 44 1610 1965
81 LAURELL, C. B Scand J Clin. Lab Invest. 6 22, 1954
82. KUNKEL, H. G and TRAUTMAN R J Clin. Invest 35 641 1956
83 MULLER EBERHARD H J Scand J Clin Lab Invest. 12 33 1960
84 JOHANSSON B G and RYMO L. Biochem J 92 5P 1964
85 SHORE, V and SHORE, B Biochem Biophys. Res Commun. 9 455 1962.
86 SHORE B Arch Biochem 71 1 1957
87 HAZELWOOD R. N J Amer Chem. Soc 80 2152 1958
88 GREEN D E and FLEISCHER S In Metabolism and Physiological Significance of Lipids Ed by R M C. Dawson and D N Rhodes Wiley New York 1964 p 581
89 VANDENHEUVEL F A Canad J Biochem 40 1299 1962.
90 ONCLEY J L. SCATCHARD G and BROWN A J Phys Chem 51 184 1947
91 MCFARLANE, A S Discuss Farad. Soc. 6 74 1949
92. BULL, H B Advances Protein Chem 3 95 1947
93 DAVIES J T and RIDEAL, E K Interfacial Phenomena Academic Press New York 1961 p 241
94 ZILVERSMIT D B J Colloid Sci 18 794 1963
95 MACFARLANE, M G and KNIGHT B C J G Biochem J 35 884 1941
96 VAN DEENEN L. L. M HOUTSMULLER U M T DE HAAS G H and MULDER, E J Pharm. Pharmacol. 14 429 1962.
97 DE GIER J and VAN DEENEN L. L. M Biochim Biophys Acta, 49 286 1961
98 EDER, H A Amer J Med. 23 269 1957
99 GOULD R G et al. J Lab Clin Med 46 372, 1955
100 ASHWORTH L. A E. and GREEN C. Biochim. Biophys. Acta 84 182, 1964
101 MACHEBOEUF M In Blood cells and plasma proteins Ed. by J L. Tullis Academic Press New York 1953 p.358
102. ZILVERSMIT D B J Clin. Invest 44 1610 1965
103 BLOMSTRAND R and AHRENS E. H J Biol. Chem 233 321 1958
104 RODBELL, M FREDRICKSON D S and ONO K J Biol Chem 234 567 1959

References

1 Nerking J *Pflüger Arch Ges Physiol 85* 330 1901
2 Haslam H C Biochem J *7* 492 1913
3 Hardy, W B and Gardner S J Physiol (London) *40* LXVIII, 1910
4 Bennhold H Ergebn Inn Med Kinderheilk *42* 273, 1932
5 Mellander O Biochem Z *277* 305 1935
6 Blix G Tiselius A and Svensson H J Biol Chem *137* 485 1941
7 McFarlane A S Biochem J *29* 660 1935
8 Jersild M and Pedersen K O Acta Pathol Microbiol Scand *15* 426 1938
9 Pedersen K O J Physiol Colloid Chem *51* 156 1947
10 Gofman J W, Lindgren F T and Elliott H J Biol Chem *179* 973 1949
11 Kunkel H G and Slater R J Proc Soc Exp Biol Med *80* 42 1952
12 Macheboeuf M Bull Soc Chim Biol *11* 268 1929
13 Cohn E S et al J Amer Chem Soc. *72* 465, 1950
14 Bernfeld P Donahue V M and Berkowitz M E J Biol Chem *226* 51 1957
15 Oncley J L Walton K W and Cornwell D G J Amer Chem Soc *79* 4666 1957
16 Burstein M Compt Rend Acad Scien *244* 3189 1957
17 Burstein M and Prawerman, A Pathol Biol (Paris) *7* 1035 1959
18 Burstein M and Samaille J Compt Rend Acad Scien *248* 3234 1959
19 Bergquist L M Carroll V P Jr and Searcy R L Lancet I 537 1961
20 Cornwell D G and Kruger F A J Lipid Res *2* 110 1961
21 Carlson L A *Clin Chim Acta 5* 528 1960
22 Hjerten S Biochim Biophys Acta *31 216* 1959
23 Cramer K and Brattsten I J Atheroscler Res *1* 335 1961
24 Gage S H and Fish P A Amer J Anat *34* 1, 1924
25 Bierman E L Gordis E and Hamlin III J T J Clin Invest *41 2254 1962*
26 Dole V P and Hamlin III J T Physiol Rev *42*·*674* 1962
27 Cornwell D G Kruger F A Hamwi G J and Brown J B Amer J Clin Nutr *9* 24 1961
28 Lindgren F T Nichols A V Hayes T L Freeman N K and Gofman J W Ann N Y Acad Sci *72* 826 1959
29 De Lalla O F and Gofman J W Meth Biochem Anal *1* 459, 1954
30 Scanu A and Hughes W L J Clin Invest *41* 1681 1962
31 Havel R J Eder H A and Bragdon J H J Clin Invest *34* 1345 1955
32 Hillyard L A, Entenman C Feinberg H and Chaikoff, I L J Biol Chem *214* 79, 1955
33 Levy R I and Fredrickson D S J Clin Invest *43* 1286 1964
34 Scanu A and Granda J L In Proc Natl congr Am chem soc 5 (Chicago 1964) p 43 c
35 Bassen F A and Kornzweig A L Blood *5* 381 1950
36 *Salt H B et al Lancet* II 325 1960
37 Fredrickson D S Altrocchi P H Avioli L V Goodman D S and Goodman H C Ann Intern Med *55* 1016 1961
38 Fredrickson, D S J Clin Invest *43* 228 1964
39 Gofman J W et al Science *111* 166 1950
40 Lewis L A et al Circulation *14* 720 1956
41 Albrink M J and Man E B Diabetes *7* 194 1958
42 Carlson L A Acta Med Scand *167* 399 1960
43 Albrink M J J Clin Invest *40* 536 1961
44 Havel R J and Carlson L A Metabolism *11* 195 1962
45 Gofman J W et al Circulation *14* 691 1956
46 Hanig M Shainoff J R and Lowy A D Jr Science *124* 176 1956
47 Gofman J W Coronary Heart Disease Thomas Springfield Ill 1959
48 Constantinides *P Experimental* Atherosclerosis Elsevier Amsterdam 1965
49 *Furman R H Howard R P Lakshmi K* and Norcia L N *Amer J Clin Nutr 9* 73 1961

105 ISSELBACHER, K. J and BUDZ, D H Nature *200* 364 1963
106 HAVEL R J In Internat conf biochem probl lipids 3 (Brussels 1956) p 265
107 FURMAN R H, HOWARD R P and ALAUPOVIC P Metabolism *11* 879 1962
108 KRUGER, F A, CORNWELL D G HAMWI, G J and BROWN J B Amer J Clin Nutr *8* 44 1960
109 ROBINSSON D S and FRENCH J E Quart J Exp Physiol *38* 233 1953
110 GORDON R S Jr BOYLE E BROWN R K CHERKES, A and ANFINSEN C B Proc Soc Exp Biol Med *84* 168 1953
111 CARLSON, L A and WADSTROM C. B Clin Chim Acta *2* 9 1957
112 MEAD J F and FILLERUP D L J Biol Chem *227* 1009, 1957
113 *GROSSMAN M I MOELLER H C and PALM L* Proc Soc Exp Biol Med *90* 106 1955
114 HAVEL, R J J Clin Invest *36* 848 1957
115 FREDRICKSON, D S McCOLLESTER D L HAVEL R J and ONO K Chemistry of Lipides as related to Atherosclerosis Ed by I H Page Thomas Springfield, Ill *1958* p *205*
116 SANBAR S S and ALAUPOVIC P Biochim Biophys Acta *71* 235 1963
117 KORN E D J Biol Chem *215* 15 1955
118 BORGSTROM B and JORDAN P Acta Soc Med Upsal *64* 185 1959
119 OLIVECRONA T George E P and BORGSTROM B Fed Proc *20* 928 1961
120 PHILLIPS G B Proc Soc Exp Biol Med *100* 19 1959
121 MARSH J B and WHEREAT A F J Biol Chem *234* 3196 1959
122 HAFT D E ROHEIM P S WHITE A and EDER H A J Clin Invest *41* 842 1962
123 RADDING C M and STEINBERG D J Clin Invest *39* 1560 1960
124 *KAY R E and ENTENMAN C J Biol Chem* *236* 1006 1961
125 CARLSON L A Acta Soc Med Upsal *65* 85 1960
126 HAVEL R J Metabolism *10* 1031 1961
127 FARQUHAR J W REAVEN G M GROSS R and WAGNER R J Clin Invest *42* 930 1963
128 AHRENS E H Jr and SPRITZ N In Biochemical problems of lipids Ed by A C Frazer Elsevier Amsterdam 1963 p 304
129 CARLSON L A and EKELUND L G J Clin Invest 42 714, 1963
130 CARLSON L A BOBERG J and HOGSTEDT B In *Handbook of physiology* Sect eds A F Renold and J F Cahill Jr Williams and Wilkins Baltimore 1965 Sect. 5 p 625
131 WALKER W J et al Amer J Med *14* 654 1953
132 *GITLIN D et al J Clin Invest 37* 172, 1958
133 LINDGREN F T NICHOLS A V and FREEMAN N K J Phys Chemistry, *59* 930 1955
134 SHORE B and SHORE V J Athero cler Res *2* 104 1962
135 LEVINE, L KAUFFMAN D L and BROWN R K J Exp Med *102* 105 1955
136 BRIVER W W RIDDLE J W and CORNWELL D G J Exp Med *110* 113 1959
137 GRUNDY S G DOBSON H L and GRIFFIN A C Proc. Soc Exp *Biol Med 100* 704 1959
138 LEVY R I LEES R S and FREDRICKSON D S J Clin Invest *44* 1068 1965
139 RODBELL M Science 127 701 1958
140 BOBBITT J L and LEVY R. S Nat meeting Am chem soc (New York *1963*) *145* Abstract 91
141 LEWIS L A QUAIFE M L and PAGE, I H Amer J Physiol *178* 221 1954
142 WOOD P IMAICHI K, KNOWLES J MICHAELIS G and KINSELL L J Lipid Res *5* 225 1964
143 NELSON G J and FREEMAN N K J Biol Chem *235* 578 1960
144 ONCLEY J L Proc Intern Sym Lipid *Transport 70 1964*
145 ONCLEY J L. and GURD F R N In 'Blood cells and plasma proteins Ed by J L Tullis Academic Press New York 1953 p 337

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ACTA MEDICA SCANDINAVICA

SUPPLEMENTUM 44

ASYMMETRY OF RENAL FUNCTION

WITH SPECIAL REFERENCE TO CHRONIC PYELONEPHRITIS

by

PER ÅKE ÖRSTEN

Accompanies Vol 180

STOCKHOLM 1966

From the IVth Medical Clinic (Renal Clinic) and the Clinical Central Laboratory
S:t Eriks sjukhus Stockholm Sweden

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ACTA MEDICA SCANDINAVICA

has been published since 1919 as a continuation of Nordiskt Medicinskt Arkiv, founded in 1869 by Axel Key The first volume of Acta Medica Scandinavica is therefore numbered LII (52)

The chief editors have been Axel Key 1869—1900, C G Santesson 1901—1915, I Holmgren 1916—1957 and Birger Strandell 1958 to date

Acta Medica Scandinavica *publishes original research papers in the field* of internal medicine Priority will be given to authors from countries which are represented on the editorial board Contributors from those countries should *submit their papers to a representative of the country in question Contributors* from other countries should send their papers to the Editor at the address below

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The annual subscription to the journal, covering two volumes, each of 6 numbers, is 140 Sw crowns or U S $27 25, *including postage*, in the Scandinavian countries and in Holland 120 Sw crowns

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ERRATA

Page 18 column 1 line 2 from bottom — for sodium, $\frac{U_{Na}}{P_{Na}}$ — read creatinine $\frac{U_{Cr}}{P_{Cr}}$

Page 31 column 2 line 7 — for Raascsou — read Raaschou

Page 36 column 2 line 2 from bottom — for patent — read patient

Page 43 *column 2 line 5 — for 00—00 — read 112—113*

Page 45 column 1 line 14 — for Na $\frac{U_{Na}}{P_{Na}}$ — read Cr $\frac{U_{Cr}}{P_{Cr}}$

Page 48 column 1 line 5 — for VIII — read VII

Page 51 column 2 line 7 from bottom — for rise — read fall

Page 71 75 77 81 Table Xa Xb column 5 — for U_{Cr} (mEq/lit) — read U_{Cr} (mg/100 ml)

Page 90 column 1 line 10 from bottom — fo 2B — read 2A

Page 105 column 1 line 7 — for ability/PAH — read ability PAH

Page 105 column 1 line 14 — for urine — read blood

Page 106 column 1 line 17 from bottom — for 55 — read 8

Page 115 column 2 line 9 and 14 from bottom — for Na — read K

Page 1?1 column 1 line 11 — for Departement — read Department

Tryckeri Balder AB Stockholm 1966

To my wife and children

CONTENTS

Translated from the Swedish

by

ERICA ODELBERG

INTRODUCTION

It is an established fact that in persons with healthy kidneys renal function is as a rule practically symmetric (Table I) This seems to be an expression of the symmetric structure of the human body Nevertheless even if the body has a symmetric structure in many respects there are several examples of asymmetry between paired organs *e g* the right and left hemispheres of the brain, and the right and left lung

A priori it could be expected that those renal diseases which involve the whole organ more or less homogeneously would not necessarily alter the symmetry of renal function On the other hand it seems plausible to assume that those renal diseases which are obviously unilateral — *e g* a tumour stricture of an artery malformation of the urinary tract or cyst — would give rise to marked asymmetry Between these two extremes we have types of renal damage in which it is questionable whether or not we can expect the symmetry of function to be affected Foremost among these diseases is pyelonephritis in which experience has shown that histologically the degree of damage may differ on the two sides irrespective of the pathogenesis

Both acute and chronic pyelonephritis are caused by pathogenic organisms, which invade the kidney in varying number Consequently in the initial stage of pyelonephritis there are always a variable number of foci of infection It seems unlikely that their spreading size and increase in number would invariably or most frequently be such that the renal damage is homogeneous and the same in both kidneys

Several workers have compared the function of the right and left kidney in both acute and chronic pyelonephritis as well as in preponderantly unilateral renal diseases Interest in the diagnosis of renal artery stenosis as a cause of arterial hypertension has widened the indications for split function tests of the kidneys A survey of previous studies on the function of each kidney separately in patients with acute and chronic pyelonephritis is given in Table II which does not however have any pretensions to completeness A considerable number of such split function tests in other renal diseases have also been described

It is evident from this table that the asymmetry in pyelonephritis has been studied by means of several parameters of renal function I have however been unable to trace any investigation in a large series in which functional tests have been made at both ureteric and renal vein catheterization

In the present investigation my main interest was focused on a comparison between the symmetry of renal extraction of para aminohippuric acid (PAH) and the symmetry or asymmetry of the renal concentration ability and the ureteric urine s content of electrolytes and bacteria In certain cases other parameters of renal function were also investigated in this respect At the Renal Clinic and the Clinical Central Laboratory S t Eriks Sjukhus these investigations among others have been made in a

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Table II *Some previous reports on the symmetry of renal function in patients with acute and chronic pyelonephritis*

Author	Year	Type of investigation										
		U_{Osmol}	Diuresis	$U_{electrolytes}$	U_{Ptt} U_{Ntt} U_{urea}	Dye tests	E_{PAH} E_D	Clearances	RPF RBF Tm	Roentgenologic exam	Radioactive renogram	Other determinations
Bergström *et al* (15)	1959						×	×	×			
Bricker *et al* (27)	1958	×						×	×			
Bumpus (46)	1931	×				×						
Dejdar (62)	1959									×		
Dejdar & Prat (63)	1958									×		
Dustan *et al* (64)	1958		×	×				×		×		
Edvall (69)	1958						×	×	×			
Fullerton (82)	1923	×	×									
Göthgen (86)	1936							×				
Hellström (95)	1931					×						
Hent (96)	1941				×							
Hradkova & Schuck (107)	1958		×	×								
Kjellbo *et al* (134)	1962	×	×	×	×			×	×			
Kusunoki *et al* (143)	1956						×					×
Michie & Michie (163)	1957							×	×			
Minder (166 167)	1929	×										×
Ockerblad (178 179)	1925											×
Ofstad (181)	1965						×					×
Olsson (182)	1962									×		
Örsten (185)	1962						×					
Parkman (189)	1965						×	×				
Poutasse *et al* (192)	1958		×	×				×		×		
Prat *et al* (195)	1958	×						×				
Richardson *et al* (210)	1965	×	×	×	×			×	×	×		
Rothauge (214)	1957							×				
Rowntree &	1910					×						
Geraghty (216 217)	1912					×						
Saheki (219)	1929	×				×						×
Schück & Hradec (223)	1958		×	×								
Schück *et al* (224)	1960		×	×								
Todd (249)	1929	×				×						
Vertes *et al* (259)	1964		×	×				×		×		
Vuust (262)	1936							×				×
Winter (270)	1956										×	

of patients with the relevant diseases at the Renal Clinic the size of the present series is limited This is chiefly because both bilateral renal vein catheterization and bilateral ureteric catheterization could not be carried out in every case

The series includes only patients who were hospitalized at the Renal Clinic, and who underwent thorough investigation there At this clinic both renal vein and ureteric catheterization are performed as routine clinical examinations in collaboration with the Clinical Central Laboratory and the Departments of Urology and Roentgenology

Initially the object of the investigation

Table I *Some previous reports on the symmetry of renal function in healthy men*

Author	Year	Type of investigation	No of invest	Symmetric function no
Boyd & Murdock (24)	1962	Radioactive renogram	10	10
Bumpus (46)	1931	Phenolphtalein app test + specific gravity	70	56
Fullerton (82)	1923	Specific gravity	117	111
Haugen *et al* (94)	1960	$C_{In} + C_{Cr} + C_{Urea} + C_{PAH}$	14	14
Heni (96)	1941	U_{NH_3}	17	13
Hradkova & Schück (107)	1958	Conc index $\frac{U_{Na}}{P_{Na}}$	8	8
Hulet *et al* (108)	1960	U_{Na}	17	13
		U_{Osmol}	17	14
		C_{In}	21	20
		C_{PAH}	17	16
		Diuresis	21	18
Magnusson (158)	1962	Radioactive renogram	5	5
Michie & Michie (162)	1951	$C_{In} + C_{PAH}$	140	98
Ofstad (181)	1965	E_{PAH}	4	3
Prat *et al* (195)	1958	Conc index $\left(\frac{U_{Cr}}{P_{Cr}} \; \frac{U_{In}}{P_{In}}\right)$	16	16
Prat & Kocvara (197)	1957	Conc index $\left(\frac{U_{Cr}}{P_{Cr}}\right)$	8	6
Rowntree & Geraghty (216)	1910	Phenolphthalein app test	7	7
Sack (218)	1940	U_{NH_3} + indigo carmine app test	5	5
Saheki (219)	1929	U_{Cr}	12	10
		U_{Osmol}	10	10
		Phenolphthalein app test	13	11
Schuck *et al* (224)	1960	U_{Na}	14	9
		U_{Cr}	14	9
Todd & Crowell (250)	1925	Phenolphthalein app test + specific gravity	31	28
Torrance *et al* (251)	1961	Radioactive renogram	19	19
Wedeen *et al* (266)	1963	Radioactive renogram	37	37
Winter (270)	1956	Radioactive renogram	5	5

large series of patients with renal diseases, especially chronic pyelonephritis Consequently, I considered that a survey of the results as regards the symmetry (or asymmetry) of renal function would be of interest as a comparison and complement to other clinical roentgenologic and anamnestic data

The diagnosis of chronic pyelonephritis is often difficult, particularly when the disease is in a latent stage I therefore regarded it as worth while to ascertain whether the results obtained could provide information of pathophysiologic interest and whether they could be of diagnostic value The present thesis comprises a *collocation* of these results I have included as comparative material some cases of other diseases affecting the kidneys such as essential hypertension stenosis of the main renal artery with hypertension chronic glomerulonephritis the nephrotic syndrome and unilateral urologic diseases In many cases both renal vein and ureteric catheterization were performed but in some patients only the latter examination was made or *vice versa*

Even if we have a fairly large material

CHAPTER I

CRITERIA OF ASYMMETRY

No sharp borderline can be drawn for what is to be denoted as asymmetry with respect to renal function. Strictly speaking asymmetry is present when the difference between the function of the two kidneys is greater than the spontaneous range of variation in each separate kidney. One can, however, scarcely expect the function to be completely symmetric, since it is known that differences normally exist between the kidneys as regards such factors as size, vascular bed and thickness of parenchyma. This implies that in order to evaluate pathologic asymmetry, a study should be made of the extent to which asymmetry exists in healthy individuals. On ethical grounds such a normal series is impracticable when analyses are to be made of urine obtained by ureteric catheterization. Consequently I have been obliged to evaluate my results in this respect by comparisons with those of other workers.

The most extensive investigation of separate renal function tests in persons without demonstrable renal disease was reported by Hulet *et al* (109). Their observations were made in 21 normotensive patients (19 women and 2 men) hospitalized at a medical clinic. A few values in their tables suggest that renal disease cannot be ruled out with certainty in every case, although these divergent values were ascribed by the authors to technical errors. The difference between the kidneys with respect to sodium concentration in urine, diuresis, osmolarity, para-aminohippuric acid and inulin clearance was calculated as a percentage of the individual mean value. Since the frequency curve of these differences showed a skew distribution, special statistical methods had to be used for calculating the normal values. Thus the difference between right and left kidney was calculated by dividing the difference Δ by the mean of the values for both kidneys. This gave a percentage difference in relation to this mean value:

$$\text{percentage difference} = \frac{\Delta}{\frac{R + L}{2}} \times 100$$

or

$$\frac{2\,\Delta}{R + L} \times 100 \quad (84)$$

According to Hulet *et al*, the mean values of the various parameters of function were higher for the left kidney than for the right. The authors did not, however, present any statistical evidence for predominance of one kidney. As far as one can judge from the values published, they do not show any significant preponderance of the left kidney.

In 5 patients in my series, bilateral renal function tests were made for suspected renal disease, but on collection of the results no pathologic processes were demonstrable in the kidneys. Despite this, I was unable to regard them as definitely healthy in this respect in view of previous symptoms from the kidneys or lower urinary tract. Consequently I have no personal normal series of subjects who had undergone ureteric catheterization. On the other hand, as far as analyses in renal vein catheterization are concerned, I have a small series as comparative

was not only to study the conditions of symmetry and diagnosis but also to study the effect on renal function of treatment of chronic pyelonephritis — particularly long term therapy — as described in several papers from the Renal Clinic and the Clinical Laboratory (42, 44, 45, 104, 183 184, 186) However, in view of the lack of beds at the Hospital, the latter task could not be carried out to a sufficient extent to merit a detailed report (*cf* Chap X, pp 112—113) Nevertheless, it is my hope that the present material will provide an adequate basis for subsequent evalution of the influence of treatment on renal function, among other matters

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$$\text{percentage difference} = \frac{\Delta}{\frac{R+L}{2}} \times 100$$

or

$$\frac{2\Delta}{R+L} \times 100 \quad (81)$$

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disease However a number of workers have shown (*cf* p 32) that asymptomatic bacteriuria occurs in up to 30 per cent of patients on admission (147) and that demonstrable chronic pyelonephritis develops in *e g* 35—40 per cent of women with asymptomatic bacteriuria during pregnancy (129) Consequently such cases cannot be ruled out with certainty in the series stated to consist of patients with healthy kidneys

In the present series the functional tests whose results were classified as denoting symmetry or asymmetry respectively were always made before instituting any therapy If the patient was taking any drug when admitted, this medication was discontinued for a week or two before the tests

Difference in Concentration Ability

Bilateral determination of the specific gravity was reported in 1923 by Fullerton (82) Such comparisons have subsequently been made in connexion with bilateral studies of the excretion of phenol red after injection of the dye (46 250) The results of these three investigations are summarized in Table I The authors gave no details of their material nor can it be inferred whether the left or the right kidney had poorer function

Among the studies made by Saheki (219) in 7 patients with healthy kidneys was concurrent determination of the specific gravity of the urine from the right and left kidney He found bilateral agreement in every case (range of variation 0 000—0 001) This range of variation was also noted in three patients with movable kidneys The test was evidently made in well hydrated subjects since the specific gravity ranged from 1 001—1 010 except in two cases (sp gr 1 026—1 025 and 1 025—1 025 on the right and left side respectively) Bumpus (46) reported that only 19 of 70 patients regarded as having healthy kidneys had identical specific gravity in the urine from both sides If on the other hand, the limit of the difference was set at 0 002 56 (80 per cent) of the 70 had the same specific gravity In the remaining 14 cases the difference ranged from 0 003 to 0 014 Since the data in the tables are confined to these 14 cases there is no possibility of calculating the percentage differences Nor is it evident whether the right or left kidney had poorer function when a discrepancy was observed Hulet *et al* (108) made simultaneous determinations of the osmolarity of the urine in 17 patients whose kidneys were evaluated as completely healthy In 14 of them the difference between the urine from the right and left side ranged from 0 12 to 7 36 per cent In three cases the difference was 16 1 17 1 and 27 0 per cent respectively The mean value of the difference was 5 81 per cent but if the three greatly divergent values are omitted the corresponding figure is 2 76 per cent Hulet *et al* stated that they were able to show an inappreciable dominance of the left kidney (see Chap II)

In view of the aforementioned observations and of personal experience I have considered it justified to set the borderline between symmetry and asymmetry at a difference of 10 per cent in osmolarity

Difference in Urinary Creatinine Concentration

A comparison between the creatinine concentration in the urine of both kidneys was made by Schück *et al* (224) in 8 subjects considered to have healthy kidneys

Table III *PAH extraction ratio in 11 healthy volunteers*

Subject no	EPAH Right Kidney	EPAH Left Kidney
1	92 4	90 0
	91 6	91 0
	93 7	90 6
		94 7
		95 6
		94 3
2	92 8	92 5
	92 1	92 8
	92 4	92 7
3	88 9	88 2
	90 3	89 0
	90 0	89 5
4	89 6	91 8
	89 3	90 4
	89 1	87 8
5	92 0	92 2
	92 3	92 4
	93 4	92 3
6	93 7	94 1
	94 3	95 0
	93 2	93 9
7	89 8	90 7
	90 4	89 8
	91 3	89 3
8	95 3	90 6
	86 3	91 4
	84 7	90 7
9	90 1	88 3
	86 1	89 3
	88 2	87 9
		88 5
		90 7
		90 7
		90 6
		89 3
10	91 3	91 3
	90 3	92 0
	92 0	91 9
11	90 0	90 9
	89 1	90 9
	88 3	90 6

EPAH mean right kidney 90 74 %
EPAH mean left kidney 91 13 %

material consisting of 11 healthy volunteers (Table III)

In the following, the difference between right and left kidney as regards the various parameters of function is expressed — as done by Hulet *et al* — as the percentage difference and not in absolute figures This is because when using a percentage difference one can, *e g* compare the kidneys with respect to various functional differences at various absolute levels Moreover, this method permits comparisons between the renal function on different occasions in the same patient, as well as comparisons between the results in different patients Difficulties arise if the comparison is based on the relation between the values for one kidney and the other, *e g right/left* (5) Thus with a given difference between the two kidneys, the relation will vary, depending on whether the percentage value is calculated on the function of the better or the poorer kidney

On the basis of these results, I have set up the criteria described in the following for renal function to be regarded as asymmetric It must however, be emphasized that these criteria are somewhat arbitrarily chosen since they are based on small series reported by several workers These investigations were, as a rule made in patients hospitalized for some other illness than renal

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Three extraction values were first determined on the right side followed by 3 or more on the left The individual *means were calculated only from the first 3 values for each* kidney

Difference *right* left kidney

$d = -0\ 23 \pm 0\ 35$
$t = 0\ 65$ No significant deviation from 0

Difference right left kidney in the same subject

$|d| = 0\ 90$ $s = 0\ 70$
95 % limits with 95 % confidence ($n = 11$)
$0\ 90 + 3\ 26 \quad 0\ 70 = +3\ 19 - -1\ 39$

Standard deviation of the values in the kidneys of the individual
Right $s = 1\ 94$
Left $s = 0\ 77$

disease However a number of workers have shown (*cf* p 32) that asymptomatic bacteriuria occurs in up to 30 per cent of patients on admission (147) and that demonstrable chronic pyelonephritis develops in *e g* 35—40 per cent of women with asymptomatic bacteriuria during pregnancy (129) Consequently such cases cannot be ruled out with certainty in the series stated to consist of patients with healthy kidneys

In the present series the functional tests whose results were classified as denoting symmetry or asymmetry respectively were always made before instituting any therapy If the patient was taking any drug when admitted this medication was discontinued for a week or two before the tests

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A comparison between the creatinine *concentration* in the urine of both kidneys was made by Schück *et al* (224) in 8 subjects considered to have healthy kidneys

Great differences were found between the two sides as regards creatinine concentration the mean value of the difference in 14 paired determinations being 21 10 per cent (range 6 06—53 66) The authors attributed this wide scattering to osmotic diuresis caused by the procedure of examination and not to any pathologic process in the kidneys

Better agreement between the kidneys was observed by Saheki (219) in an investigation of 12 persons with healthy kidneys He stated that the mean value of the difference between the creatinine concentration in the urine from either side was 9 10 per cent (range 0—22 22)

Chiefly on the basis of Saheki s results I have drawn this borderline at a difference of 20 per cent between the kidneys

Difference in Urinary Sodium Concentration

To investigate the symmetry of the urinary sodium concentration Schuck *et al* (224) made 14 bilateral determinations in 8 subjects with healthy kidneys and lower urinary tract They noted a mean difference of 10 43 per cent (range 1 34—22 22) Hulet *et al* (108) found a corresponding mean value of 6 99 per cent in their series of 17 healthy subjects Four values nevertheless deviated greatly from the others *i e* 10 0 15 5 16 0 and 26 7 per cent The mean difference when these were omitted was 3 89 per cent (range 1 48—8 80)

In view of the observations of Hulet *et al* I have somewhat arbitrarily set the limit of symmetry at a difference of 10 per cent However taking into account some previous reports one must anticipate occasional greater asymmetry even in subjects with healthy kidneys

Difference in Urinary Potassium Concentration

I have been unable to find any data in the literature regarding the symmetry of this parameter of renal function in persons with healthy kidneys In analogy with the osmolarity and sodium concentration the borderline has therefore been drawn arbitrarily at a difference of 10 per cent

Difference in Diuresis

In the series of Hulet *et al* (108) the mean difference between the diuresis of the two kidneys in 21 subjects with intact renal function was given as 5 17 per cent (range 0 00—26 3) When the three most deviating values (26 3 15 2 and 10 3) were excluded the mean value was 3 16 per cent (range 0 00—8 17) In my series the diuresis of the two kidneys separately was determined only in patients with renal artery stenosis with arterial hypertension in addition to two patients with essential hypertension The borderline for asymmetry was therefore drawn at a difference of 50 per cent between the kidneys in accordance with the values of several authors (56 57 106 220 276)

Difference in pH of Urine

The ammonia and titratable acid content of bladder and ureteric urine was determined in many cases in the present series (*cf* Chap VII) Since for various reasons the determinations were not made consistently these values have not been listed in the tables The pH was also determined but this test on the ureteric urine was not as a rule preceded by acidification partly because the patient was deprived of fluids until the urinary concentration test was made concurrently

The pH was therefore regarded to be of inappreciable value as a basis for evaluating the acidifying ability of one kidney in relation to that of the other The total acidifying ability of the kidneys was on the other hand determined after an ammonium chloride load

Sack (218) stated that the ammonia concentration was identical in the urine from both kidneys and Heni (96) demonstrated that the difference did not exceed 10 per cent in subjects with healthy kidneys As far as I have been able to ascertain, the literature contains no corresponding data on the pH of the urine However since the error of the method is exceedingly small I have set the borderline for the difference in pH at 0 05 Obviously this difference cannot be calculated as a percentage

Difference in Clearances

Separate *endogenous creatinine clearances* were calculated in clinically healthy patients by *e g* Haugen *et al* (94) in their 14 cases they found good agreement between the kidneys in this respect This also applied to a patient with chronic glomerulonephritis The greatest difference noted was 5 ml min corresponding to 6 per cent

Haugen *et al* (94) also determined the *inulin and para aminohippuric acid (PAH) clearance* in the ureteric urine of the same 14 subjects Here as well there was good agreement with no difference between the kidneys for inulin and a mean difference of 2 5 per cent for PAH

Hulet *et al* (108) made 2? C_{In} determinations in the urine of each kidney in their aforementioned series The mean of the differences between the kidneys was 7 59 per cent (range 0 37—21 2) In 17 determinations of C_{PAH} the corresponding mean was 6 34 per cent (range 0 32—20 2)

Michie & Michie (162) also compared these two parameters in 110 determinations (number of subjects not stated) In more than 70 per cent, they found that the inulin and PAH clearance was almost identical for both kidneys They did not present any tables or data regarding the results in the remaining 30 per cent of the determinations In a study of patients with renal tuberculosis Hoeg (102) expressed the view that the sources of error in determining the urinary clearance of urea inulin endogenous creatinine and PAH do not permit greater accuracy than ± 5 per cent

Since I have no series of healthy subjects I have relied entirely on the aforementioned data with respect to clearance determinations as well I have set the borderline for asymmetry at a difference of 20 per cent between the kidneys

Difference in Renal Plasma Flow

I have not succeeded in tracing any reports of studies on the difference in renal plasma flow (RPF) between the two kidneys of healthy subjects Consequently in analogy to the differences in clearance the borderline has been fixed at 20 per cent

Difference in Para Aminohippuric Acid Extraction

The literature contains a number of data on normal values for the total para aminohippuric acid (PAH) extraction (14 25 33 47 143 208 232 265) As rule this has been calculated on the right kidney Bucht (41) reported the results of 29 renal vein catheterizations made on the right kidney with a

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the difference was counted This grading is in conformity with bacteriologic practice (cf Chap VII)

Difference in Certain Clinical Features

Obviously such clinical features as a feeling of tension pain in the flank and tenderness to palpation over one or both loins are highly subjective criteria They are however of considerable value not only for the diagnosis of pyelonephritis in general but also for establishing the predominance of the disease on one side or the other Consequently the existence of a difference between the kidneys in this respect was compared with that of asymmetry in the functional tests

mean PAH extraction of 87 9 per cent (range 84—96), and 8 tests in other healthy subjects, in which the mean extraction by the left kidney was 87 4 per cent (range 83—91)

Ofstad (181) reported determination of the E_{PAH} by the right and the left kidney in 4 subjects with normal renal function In 3 cases, the difference ranged from 2 20—3 30 per cent In the remaining case, the difference between the kidneys was 9 09 per cent, with a mean of 4 24 per cent for all tests Since, as far as I am aware, no other simultaneous study has been made of the PAH extraction by the two kidneys separately, I made this test in 11 healthy subjects The mean extraction by the right and the left kidney was 90 7 and 91 1 per cent, respectively (*cf* Table III) On the basis of these results, I set the borderline for asymmetry between the kidneys with respect to PAH extraction at 3 per cent

Roentgenologic Examination

Cases have been denoted as roentgenologically asymmetric only when unquestionable differences between the kidneys were visible on the films When evaluating these differences, I have relied exclusively on the statements of the roentgenologist who studied the films of the upper urinary tract of all the patients without knowledge of the other data in these cases

Evaluation of differences between the kidney and ureter of either side was based largely on the criteria of Dejdar (62) Dejdar & Prat (63) and Olsson (182) On the plain films, the kidneys were compared with respect to size and shape sharpness or irregularity of the contours and presence of concretions if any The excretory pyelograms were studied to demonstrate differences between the thickness of the renal parenchyma, dilatation of the pyeloureteric system, and deformation of the calyces, *e g* papillary atrophy and destruction, and rounding of the fornices Delayed or poor excretion of contrast medium and density of the medium were also evaluated

As comparative material, I included cases of renal disease in which involvement was regarded as homogeneous, and in which roentgenologic examination did not disclose any difference between the two kidneys

In addition, patients with preponderantly unilateral renal disease were investigated In some of the latter cases, examination disclosed developmental anomalies, concretions in the renal pelvis hydronephrosis or hydroureter, sometimes caused by ureteric concretions, stricture or reflux In this group the difference between the two sides was easier to detect If functional changes were present — *e g* variations in tonus in the upper urinary tract — and affected the roentgenologic features they were not taken into account

Renal angiography was performed in hypertensive patients with suspected stenosis of the main renal artery as well as in pyelonephritis and other renal diseases to provide a more all round picture of such features as size of the kidney and the existence of scarring In addition retrograde pyelography and micturition cystography were done in some cases partly to illustrate the asymmetric involvement by the pathologic process

Difference in Bacterial Count

When the number of bacteria in the urine from one kidney was more than 10 times as great as that in the urine from the other

who had died in accidents or by violence and demonstrated that the kidneys decrease in size after 50 years of age

Roentgenologic studies have also been published which confirm the aforementioned anatomic differences between healthy kidneys (168 188 222) The most extensive investigation of this kind hitherto reported is that of Moëll (169) who measured the length, width and area of both kidneys in 100 healthy men and 100 healthy women The mean values we e as follows

MEN	Right kidney	Left kidney
Length × width (cm)	12 9 (s = 0 80) × 6 2 (s = 0 45)	13 2 (s = 0 79) × 6 3 (s = 0 49)
Area (cm²)	79 6 (s = 8 75)	82 7 (s = 8 34)
WOMEN		
Length × width (cm)	12 3 (s = 0 79) × 5 7 (s = 0 46)	12 6 (s = 0 77) × 5 9 (s = 0 42)
Area (cm²)	70 1 (s = 8 00)	74 1 (s = 7 31)

Moëll concluded from these figures that both the right and left kidney are significantly larger in men than in women and that the left is larger than the right in both sexes

As mentioned in Chapter I Hulet *et al* (108) claimed to have found a functional dominance — even if it was inappreciable — of the left kidney this was not however demonstrated statistically Nor did they demonstrate any sex difference with respect to renal function

The authors who have studied the various functions of both kidneys in healthy subjects and the incidence of symmetry reported by them are listed in Table I The table has no pretensions to completeness

CHAPTER II

EARLIER BILATERAL COMPARISONS BETWEEN HEALTHY KIDNEYS

In the previous chapter, I gave an account of the criteria of differences between the two kidneys with respect to the parameters of renal function applied in the present study In the following, a brief survey is given of some earlier investigations on the symmetry of healthy kidneys as regards both function and anatomy

As early as 1893, Suter & Meyer (246) reported determinations of the *diuresis* from each kidney in a healthy 5 year old boy The urine was collected from the ureteric orifices which opened into an ectopic bladder During a 6 hour period, the excretion of urine, urea and phosphoric acid was approximately the same from both kidneys The difference in diuresis during the 3½ days duration of the test was 4 5 per cent the lower value being noted for the left kidney

It was not until Nitze of Vienna introduced a practicable cystoscope in 1877 (48) that the prerequisites were created for catheterizing the ureters The first properties to be investigated were the urinary output, specific gravity of the urine and its content of various metabolites and electrolytes (*e g* urea creatinine and sodium), and the excretion of certain dyes

The tubular reabsorption of water was studied by Hradkova & Schuck (107) with the help of the *concentration index* of sodium, $\frac{U_{Na}}{P_{Na}}$ They found no marked difference in this respect between the two kidneys of children with normal renal function

Prat *et al* (195) calculated the *concentration index* of the urine from each kidney and found good bilateral agreement They defined the concentration index as the ratio of the creatinine or inulin in urine to that in plasma

Many studies of renal function are based on determinations of the rate of excretion by the kidneys of a dye administered intravenously or orally As early as 1910, Rowntree & Geraghty (216) found good conformity between the kidneys of healthy subjects with respect to the excretion of *phenol red*

In recent years *renograms* have been suggested as a general screening test of the symmetry of renal function They were first described by Winter (270) who stated that identical renograms of both kidneys could be observed when renal function was normal This was confirmed by later workers (24 158, 251 266)

Several authors have described *anatomic* comparisons between the kidneys Thus in 1872 Pourteyron (191) made a post mortem examination of 86 pairs of kidneys and found that the left was slightly larger in both sexes Moreover both kidneys were somewhat smaller in women than in men and their total weight was lower The left kidney has also been found to weigh more than the right (148 191) De Leon (148) made post mortem studies in 768 persons

teria or anamnestic data may have been lacking

Obviously I took into account a number of symptoms and signs as well as those listed as cardinal criteria. A diagnosis of chronic pyelonephritis was not, however, made unless at least three of them were fulfilled, irrespective of which other renal symptoms were present. This also applied to the cases in which all eight criteria could not be tested. In a few patients who did not fulfil even three criteria, the diagnosis was nevertheless considered as highly probable on the grounds of other symptoms and signs. They are accounted for in a separate group (A2) denoted as acute or suspected chronic pyelonephritis.

In addition to the diagnostic criteria described in detail in the following and the patients' statements, attention was also focused on the existence of, *e.g.*, concretions or malformations of the urinary tract, hypertrophy of the prostate, diabetes mellitus and other complicating diseases. These are listed in Table IV. For the classification of the material reference is made to Chapter V.

Naturally, one cannot rule out with certainty the existence of chronic pyelonephritis in individual patients in the other groups of renal disease investigated as comparative material.

The reverse — *i.e.*, the existence of another renal disease dominated by superimposed chronic pyelonephritis — can also apply. The coexistence of chronic pyelonephritis and renal tuberculosis was reported by, *e.g.*, Jenni (115). My series contains two such cases (nos. 113, 114). They were not included in the chronic pyelonephritis group, even though they fulfilled the criteria. In recent years chronic glomerulonephritis has become less common in Sweden (106). For this reason among others it is highly improbable that any patient with silent glomerulonephritis and superimposed pyelonephritis would have been included in the present series. In two cases (nos. 108, 116) this possibility could not, however, be definitely ruled out, and they were not therefore assigned to the chronic pyelonephritis group.

The clinical features and results of examinations were divided into the following four main groups:

A. Renal symptoms or results of renal function tests which, at any rate when present concurrently, strengthen the suspicions of chronic pyelonephritis — denoted here as *cardinal criteria*.

B. General symptoms of renal disease.

C. General signs of renal disease.

D. General signs of disease.

A. Cardinal Criteria

1. *Certain anamnestic data*

Among these data were *dysuria* and/or *pollakiuria* either at the time of examination or in the history. Anamnestic data on these symptoms were taken into account only if they had been confirmed by a physician and had been associated with pyuria and/or bacteriuria. The coexistence of *fever, pain* and a feeling of *tension in the flank* was a strong reason to conclude that the aforementioned complaints were not merely signs of infection or irritation of the lower urinary tract (cystitis and/or urethritis) but that the renal tissue was, in fact, involved. An attack of *hyperpyrexia* without local symptoms and signs was, on the other hand, counted as a sign of acute pyelonephritis only if massive pyuria and bacteriuria were demonstrable concurrently. Brod (29) found in his

CHAPTER III

DIAGNOSIS OF CHRONIC PYELONEPHRITIS

It is often difficult to establish an unquestionable diagnosis of chronic pyelonephritis, and in almost every case there is in fact, some degree of uncertainty In many cases in which the diagnosis is regarded as fairly incontrovertible, one or more of the clinical features or results of tests usually considered as significant are lacking On the other hand none of the most characteristic features is pathognomonic of chronic pyelonephritis Consequently, when a differential diagnosis is to be made between chronic pyelonephritis and other renal diseases one can seldom avoid some degree of subjectivity in the evaluation In order to disclose as many anamnestic data as possible, and to permit uniform evaluation of the case material all patients with suspected chronic pyelonephritis and other renal diseases who were admitted to the Renal Clinic of our hospital had therefore, to reply to a detailed questionnaire

On the basis of the data obtained in this way thorough physical examination and various functional tests I set up eight cardinal criteria of chronic pyelonephritis According to other authors (10a and b, 28 29, 30, 31, 37 38 135, 194) and personal experience in a large series of cases these features are particularly common in the disease in question When at least three of these criteria were unquestionably fulfilled the patient was denoted as having chronic pyelonephritis These eight cardinal criteria were

1 A history of dysuria and or pollakiuria combined either with fever and pain in the flank, or with pyuria and or bacteriuria
2 Tenderness to palpation over one or both loins
3 Decreased ability to acidify the urine
4 Reduced renal concentration ability
5 Characteristic interstitial changes at histologic examination of renal tissue
6 Bacteriuria
7 Characteristic appearance of the urinary sediment
8 Characteristic roentgenologic changes in the kidney and renal pelvis

It must be stressed once more that none of these symptoms or signs singly can be regarded as characteristic of chronic pyelonephritis but that the diagnosis is strongly supported if they are present concurrently Thus dysuria alone combined with a feeling of tension in the flank does not suffice for a diagnosis If however bacteriuria also exists the first mentioned feature carries much more weight According to experienced workers, even the microscopic features of a biopsy specimen may occasionally be misleading (112) In 24 of my 80 cases of chronic and acute non obstructive pyelonephritis (groups A1 A2) the occurrence of all eight cardinal criteria could not be investigated For example a renal biopsy may have been unsuccessful it may have been impossible to determine the acidifying ability of the urine in cases with urea splitting bac

Case	Haematuria	Cloudy ill smelling urine	Pregnancies (no)	Phenacetin abuse	Previous bladder catheterisation	BP (mm Hg)/24 hours or on admission	BP (mm Hg)/24 hours (min)	Ocular fundi (Keith & Wagener) FH	Serum creatinine (mg/100 ml)	Water loading test (mOsmol/lit.)	Proteinuria	Clearances ml min 1 73 m BSA: PAH	Inulin	Endog creatinine	General signs of disease	Intercurrent diseases
	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32
GROUP A1 Chronic non obstructive pyelonephritis (≥ 3 cardinal criteria)																
	0	+	III	0	+	130 85	105/75	I	1 8	140	(+)	(270)	(53)	(49)	+	
	0	0	II	–	0	210 140	160 100	I II	1 8	195	++	211	5_	49	+	Art. hypert.
	+	–	III	0	–	170 110	130 80	II	1 6	94	(+)	136	–	49	+	Art. hypert
	+	0	I	0	+	1 0 80	90 60	0	2 0	97	(+)	(148)	(31)	(4)	+	
						120 75	90 50	0	1 7	75	(+)	(49)	(52)	(51)		
	+	0	1	0	0	160 110	130 90	0	2 8	118	+	(61)	(14)	(22)	+	Art. hypert
	0	0	0	0	0	175/90	105 65	0	2.1	281	(+)	275	–	32	+	
						110 65	—	0	2.2	170	(+)	(117)	(29)	(70)		
	0	+	III	0	0	150 100	130 85	I II	0 9	85	0	373	–	130	+	Art. hypert.
						150 90	170 70	0	0 9	75	0	290	–	80		
	0	0	II	+	+	185/100	155 85	0	1 6	107	0	(404)	(87)	(60)	+	
						230 115	155 90	II	2.3	204	(+)	(167)	(40)	(44)		Art. hypert.
a	+	+	0	(+)	0	110 65	140 80	0	1.2	108	0	(46)	(12)	(5)	+	
b						145 80	—	1	2.1	100	0	(141)	(30)	(39)		
	0	0	0	0	0	140 110	105 85	0	0 8	69	0	—		—	+	Art. hypert
			I	–	0	110 70	85 55	0	4.2	163	+	(73)	(17)	(23)	+	
	+		0	–	–	130 80	—	–	1 1	84	0	(304)	(78)	(57)	+	
3a	0	0	II	(+)	0	115 90	105 80	0	1.3	175	(–)	—		—	+	
3b						140 80		I	1 4	90	0	(403)	(74)	(72)		
4b	0	0	0	0	0	125 75	105 55	I	2.6	105	+	94	–	_6	+	
4c						145 90	110 70	0	1 8	90	0	(198)	(35)	(53)		
	0		1	+	0	165 95	170 70	0	1 9	85	(+)	(291)	(55)	(56)	+	
6			III	+	0	170 110	145 90	0	2 0	113	(+)	706	(35)	39	+	Art. hypert.
7		+	0	+	+	180 80	105 65	0	1 8	85	(+)	—	(23)	(34)	+	Bl. renal papill. necr
18			IV		0	160 110	140 80	—	1 9	117	(+)	171	—	29	+	
19a	0	0	I	+	0	30 125	195 100	I	2 3	117	(+)	121	(27)	41	+	Art. hypert., Bl. renal papill. necr
19b						30 100	190 90	II	2 6	150	(+)	204	(27)	33		
20a				(+)	+	25 140	165/130	II	1 9	—	(+)	165	(85)	63		Art. hypert., Bl. renal papill. necr
20b						150 100	170 75	I	1 8	305	—	67	—	64	—	
1			0			160 100	—	III	1 4	—	0	—	—	—	+	Art. hypert., Bil. renal papill. necr
a			II		0	145 85	1 5/75	0	2 6	9	(+)	179	—	47	+	
b						155 90	130 75	–	2.2	75	+	—		—		
23	0		II	0		115 75	100 60	0	1 0	75	0	(616)	(115)	(93)	0	
24	0		III		+	1 5 75	105 60	0	2.0	265	(+)	18	38	35	+	Bil. renal papill. necr
	0	0	III	–	–	145 80	—	0	1 ?	75	(+)	330	—	103	+	
25b						140 85	1_0 70	0	1.2	60	0	3 1		8		
6	0	0	0		+	1 0 85	105/75	0	1 3	90	0	371	(56)	48	+	
6b						1 5 80			1 8	100	(+)	386	(83)	6		
7			0			95 65	85 45	0	1 1	25	(+)	(51)	83	76	–	
9	0		II		+		—	–			0	46	–	116	+	Art. hypert.
8b						170 110	150 95	0	1 8	207	0	—	–	–		
8c						10 130	190 170	0	2.7	145	+	(98)	(_6)	(40)		
29a	0	0	II	0		160 90	—	I II	1 3	60	0	441		97	+	
29b						195 95		II	_8	—	(+)	14	–	33		
29c						180 105	145 60	II		105	0	(19)	(40)	(50)		Art. hypert.
30a	0	0	II	0	0	160 105	105 70	0	1 6	4	(+)	(601)	(1_8)	(65)	+	Diab. mell
30b						150 95	105 75	–	1 7	170	++	(574)	(49)	(80)		Art. hypert
31		0		0	0	130 85	105 70	0	1 3	75	0	3 4	—	115	+	
3	0		1			180 110	145 95	0	1 3	60	(+)		—	—	+	Art. hypert., Bl. renal papill. necr
33	+		0	–		190 95	130 90	I	0 8	94	0	34.	—	..		
34	+		0	+	+											

Table IVa Cardinal criteria and salient data
Symbols as in Table IVb

Case no	Case Record no	Age at invest (yrs)	Sex	Cardinal criteria of pyelonephritis: Cystitis without fever	Cystitis with fever	Chills without cystitis	Hist of loin pain	Loin tenderness to palpation	Ac dif abil (pH)	Max conc ability (mOsmol/lit)	Histologic exam B = Biopsy R = Resection or ectomy A = Autopsy	Bacteriuria no/ml	Urinary sediment	Roentgenol exam	
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	
GROUP A1 Chronic non obstructive pyelonephritis (≥ 3 cardinal criteria)															
1	2261/60	41	F	0	+	0	+	0	5 36	535	R +	700 000 Ec	−	+[1]	
2a	9952/58	38	F	0	+	0	+	+	4 98	388	−	26 mill Ec.	−	+	
3	2593/62	50	F	−	+	−	+	0	5 54	465	B 0	500 000 Ec	+	+	
4a	7655/65	45	F	+	+	+	+	0	5 4	313	B +	10 mill. Ec	+	+	
4b		47							5 68	520	−	1 mill Ec.	+	+	
5	†4449 60	31	F	+	0	0	0	0	5 2	369	A +	51 mill Ec	+	+	
6a	7106/61	43	F	0	0	+	+	+	5 1	—	B 0	16 mill Ec	+	+	
6c		46							5 75	500	−	0	0	−	
7a	4710 61	66	F	+	+	0	(+)	0	5 13	390	B +	24 mill Ec	+	0	
7b		68							5 15	577	−	0	0	0	
8a	1415/61	49	F	+	0	0	0	0	4 96	412	B +	10 5 mill Ec	+	0	
8b		52							5 60	467	−	0	0	+	
9a	4413 61	57	F	+	+	+	+	0	5 15	354	B +	>100 000 Ec	+	−	
9b		60							5 25	534	−	0	0	+	
10	9141/58	22	F	+	0	0	0	0	5 3	747	B +	96 5 mill. Ec	−	0	
11	7592/58	38	F	−	−	(+)	+	+	5 0	317	B 0	19 mill Ec	+	0	
12	9317/58	55	F	−	+	−	+	0	5 0	254	B 0	>100 000 Ec	+	+	
13a	7467/61	55	F	0	+	+	0	0	5 05	250	−	75 mill Ec.	+	+	
13b		58							5 02	620	−	0	0	+	
14b	540 61	26	F	0	0	+	(+)	0	5 35	505	B +	68 mill Aa	+	+	7
14c		28							5 56	573	−	0	0	−	
15	5243 61	51	F	+	+	0	+	+	5 46	422	B +	10 mill Ec.	+	+	8
16	4655 61	62	F	+	+	0	+	0	5 20	430	B +	120 mill Ec	+	+	6
17	9915/59	35	F	+	+	0	+	0	5 56	415	B +	75 mill Ec.	+	+	7
18	229 60	65	F	+	+	0	+	+	4 93	395	B 0	90 mill Ec	+	+	6
19a	†6401/63	55	F	+	+	0	+	0	5 9	409	B +	15 mill Ec	+		7
19b		58							5 49	395	A +	0	0	+	
20a	8471/62	50	M	+	+	−	+	0	−	—	−	>100 000 Aa	+	+	5
20b		53							−	480	−	34 mill Aa	−	+	
21	4391/62	45	F	0	0	0	0	+	−	—	−	60 mill Ec.	+	+	4
22a	8536/61	30	F	0	+	0	+	0	5 4	352	B +	>100 000 Ec	−	−	7
22b		33							5 5	365	−	20 mill. Ec.	−	−	
23	8198/61	41	F	+	0	0	0	0	5 51	930	B +	73 mill Ec	+	−	6 8
24	1717/59	45	F	+	+	0	+	+	5 15	350		7 mill Ec	+	−	6
25a	1086/62	44	F	+	0	+	+	0	5 50	338	B +	>100 000 Ec.	−	+	7 8
25b		46							8 12	450		18 mill Pm	+		
26a	6423 63	45	F	+	+	+	+	0	5 65	390	B 0	300 000 Ec	−	0	6 8
26b		46							6 33	505	−	300 000 Ec			
27	9600 59	21	F	+	+	+	+	+	5 05	865	B 0	10 mill Ec.	+	0	4 8
28a	†6940 62	31	F	0	+	0	+	0		—	−	>100 000 Ec	0	−	
28b		31							5 6	—		0	0		6 8
28c		32							4 74	395	A +	0	0		
29a	445 62	52	F	+	+	+	+	+	5 6	455	B (−)	0	0		
29b		54							−	—	−	25 mill Pv			7 8
29c		55							5 0	513	−	0	0		
30a	1868 61	30	F	0	0	+	+	+	5 45	450	B 0	>100 000 S alb	+		7 8
30b		34							5 20	5 0	−	0	0		
31	7739 61	48	M	0	+	0	+	+	5 05	765	B 0	>100 000 Aa	0	0	4 8
32	6606 62	40	F	0	+	0	+	+	5 45	475	B +	1 mill Ec	−		8 8
33	1835/62	54	F	0	+	0	0	+	4 65	761	B 0	>100 000 Ec	+	0	5 8
34	5846 63	66	F	0	+	0	(+)	+	6 89	565	B +	190 mill Pm	+		8 8
35	†4165/62	43	F	0	0	0	0	0	5 20	355	B A +	0		−	3 7

[1] Left kidney

Case no	Haematuria	Cloudy ill smelling urine	Pregnancies (♀)	Phenacetin abuse	Previous bladder catheters	BP (mm Hg) 24 hours (max) or on admission	BP (mm Hg) 4 hours (min)	Ocular fundi (Keith & Wagener) FH	Serum creatinine (mg/100 ml)	Water loading test (mOsmol/l)	Proteinuria	Clearances ml/min 1.73 m² BSA PAH	Inulin	Endog creatinine	General signs of disease
1	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31

GROUP AI Chronic non-obstructive pyelonephritis (≥ 3 cardinal criteria)

1	0	+	III	0	+	130 85	105 75	I	1 8	140	(+)	([illegible]70)	(53)	(49)	+
2	0	0	II		0	220 140	160 100	I II	1.8	195	++	211	5	49	+
3	+		III	0	–	170 110	130 80	II	1 6	94	(+)	136	–	49	+
4a		0	I	0	+	1 0 80	90 60	0	2.0	97	(+)	(148)	(31)	(–4)	+
4b						120 75	90 50	0	1 7	75	(+)	(249)	(5)	(51)	
5	+	0	I	0	0	160 110	130 90	0	2.8	118	+	(61)	(14)	(––)	+
6a	0	0	0	0	0	125 90	105 65	0	2 1	–87	(+)	225	–	3	+
6c						110 65		0	2 2	170	(+)	(117)	([illegible]9)	([illegible]0)	
7a	0	–	III	0	0	150 100	130 85	I II	0 9	85	0	373	–	130	+
7b						150 90	120 70	0	0 9	75	0	290	–	80	
8	0	0	II	+	+	185 100	155 85	0	1 6	107	0	(404)	(87)	(60)	+
8b						230 115	155 90	II	3	204	(+)	(167)	(40)	(44)	
9a		+	0	(+)	0	110 65	140 80	0	1 2	108	0	(46)	(17)	(–5)	+
9b						145 80		1	2 1	100	0	(141)	(30)	(39)	
10	0	0	0	0	0	140 110	105 85	0	0 8	69	0	–	–	–	+
11			1	–	0	110 70	85 55	0	4 2	163	+	(73)	(17)	([illegible]3)	+
1			0	–		150 80	–	–	1 1	84	0	(304)	(78)	(57)	+
13a	0	0	II	( )	0	115 90	105 80	0	1 3	175	(+)	–	–	–	+
13b						140 80		I	1 4	90	0	(403)	(74)	(72)	
14b	0	0	0	0	0	1 5 75	105 55	1	2.6	105	+	94	–	–6	+
14c						145 90	110 70	0	1 8	90	0	(198)	(35)	(53)	
15	0		1		0	165 95	1–0 70	0	1 9	85	(+)	(291)	(55)	(56)	+
16			III	+	0	170 110	145 90	0	2 0	113	(+)	–06	(35)	39	+
17			0	+		180 80	105 65	0	1 8	85	(+)	–	(3)	(34)	+
18			IV		0	160 110	140 80		1 9	117	(+)	171	–	29	+
19a	0	0	I	–	0	30 1–5	195 100	I	–3	117	(+)	121	(27)	41	+
19b						230 100	190 90	II	2.6	150	(+)	–04	(27)	33	
20a				( )		–25 140	165 130	II	1 9		(+)	165	(85)	63	
20b						150 100	170 75	I	1 8	305	–	267	–	64	–
1			0			160 100	–	III	1 4	–	0	–	–	–	+
–a			II		0	145 85	125 75	0	6	9	(+)	179	–	47	+
b						155 90	130 75	–	–	75	+			–	
3	0		II	0		115 75	100 60	0	1 0	75	0	(616)	(115)	(93)	0
4	0		III		+	1 5 75	105 60	0	2 0	65	(+)	–18	38	35	+
a	0	0	III	+		145 80	–	0	1–	75	(±)	330	–	103	+
5b						140 85	120 70	0	1	60	0	321	–	8–	
6a	0	0	0			1 0 85	105 75	0	1 3	90	0	3 1	(56)	48	
6b						1 3 80	–		1 8	100	(+)	486	(83)	6	+
7			0			95 65	85 45	0	1 1	25	(+)	(512)	83	76	
5	0		II				–				0	46	–	116	+
8b						170 110	150 95	0	1 8	207	0	–	–	–	+
8c						10 130	190 170	0	–7	145	+	(98)	(6)	(40)	
9a	0	0	II	0		160 90		I II	1 3	60	0	441		97	
9b						195 95		II	–8	–	(+)	14–	–	33	+
9c						180 105	145 60	II	–	105	0	(19)	(40)	(50)	
30a	0	0	II	0	0	160 105	105 70	0	1 6	4	(+)	(601)	(1–8)	(65)	
30b						150 95	105 75		1 7	170	++	(574)	(59)	(80)	+

Table IVa (continued)

Case no	Case Record no	Age at invest (yrs)	Sex	Cardinal criteria of pyelonephritis: Cystitis without fever	Cystitis with fever	Chills without cystitis	Hist of loin pain	Loin tenderness to palpation	Acidif abil (pH)	Max conc ability (mOsmol/lit)	Histologic exam B = Biopsy R = Resection or ectomy A = Autopsy	Bacteriuria no /ml	Urinary sediment	Roentgenol exam	No pos criter a/No criteria tested
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
							GROUP A1 (continued)								
36	1303/61	49	F	+	+	0	+	0	5 07	802	B 0	>100 000 Ec.	+	0	3 8
37	8837/61	35	F	+	+	0	+	0	5 45	680	B 0	4 mill Ec	0	0	4 8
38	4927/61	39	M	0	+	0	+	+	5 10	514	B +	3 mill. Ec	+	+	7 8
39	† 405/64	31	F	+	0	0	0	0	5 10	385	A +	0	0	0	3 8
40	3230 62	51	F	0	+	0	+	+	–	—	B +	>100 000 Ec.	+	+	6 6
41	9412/60	45	F	0	(+)	+	0	0	5 34	570	B +	900 000 Ec	+	+	7 8
42	7672/62	54	F	0	+	0	+	0	4 75	675	B +	>100 000 Ec.	+	0	5 8
43	2487/61	58	F	0	+	0	+	+	4 75	416	B +	41 mill Ec	+	0	6 8
44	1604/63	27	F	+	0	0	(+)	0	5 68	345	B +	0	0	+	5 8
45	3010 61	48	F	+	0	0	0	0	–	902	B +	0	+	+	4/7
46	45/60	37	F	0	(+)	+	+	+	5 3	345	—	8 mill Ec.	+	+	7/7
47	1453/60	60	F	0	+	+	+	0	5 22	475	—	15 mill Ec.	+	0	4/7
48	8666/62	18	F	+	0	0	+	0	4 91	1100	B 0	>100 000 Ec.	+	0	3 8
49	6974/62	24	F	0	+	0	+	+	–	950	B +	>100 000 Ec.	0	0	4/7
50	6972/62	49	M	0	+	0	+	0	–	—	B +	3 4 mill Ec.	+	+	5 6
51	6474/62	42	F	0	+	–	+	+	–	430	—	1 2 mill Ec.	+	+	6 6
52	2227/63	54	F	+	0	0	0	0	–	600	B +	0	0	+	4/7
53	6879/62	56	F	+	0	0	+	+	5 47	840	—	135 000 Pm	–	0	5 7
54	2869/61	41	F	+	0	–	0	0	5 75	410	B +	4 mill Ec	+	+	7 8
55	2409/62	24	F	0	+	–	+	+	5 25	945	B 0	>100 000 Ent.	0	–	5 8
56	2962/62	46	F	0	+	0	+	+	–	395	B +	40 mill Ec.	+	+	7/7
57	4681/61	54	F	0	+	0	+	0	5 10	725	B 0	>100 000 Pv	+	0	4 8
58	2960 62	46	F	0	+	0	0	0	4 75	675	—	2 mill Ec	+	+	5 7
59	6632/62	42	F	+	+	0	+	+	6 51	345	—	>100 000 Ec.	–	+	7 7
60	6412/62	34	F	+	+	0	+	+	5 12	458	B +	1 mill Ec.	0	+	6 8
61	3550/59	53	F	+	+	0	+	0	5 3	370	B +	>100 000 Ec	+	+	7 8
62	2406/59	43	F	+	+	0	+	+	5 3	535	B (+)	60 mill Ec.	+	0	7 8
63	4070/64	55	F	0	+	0	+	+	5 05	545	—	600 000 Ec.	+	0	5 7
64	5416/59	35	F	0	0	0	0	0	5 4	535	—	60 mill. Ec	+	+	5 7
65	7872/61	34	F	0	+	+	+	+	5 0	492	B 0	>100 000 Ec	+	+	6 8
66	4358/59	52	F	+	0	0	0	0	5 3	470	B +	800 000 Ec.	–	+	7 8
67	2598/62	41	F	0	+	0	+	+	4 65	670	B +	>100 000 Ec.	–	0	6 8
68	1599/64	67	F	0	+	+	+	0	5 00	835	B +	110 mill. Pv	–	0	4 8
69	359/65	44	F	+	0	0	0	0	6 56	390	B +	0	–	0	5 8
							GROUP A2 Acute and suspected chronic non obstructive pyelonephritis (< 3 cardinal criteria)								
70	901/60	35	F	+	0	0	+	0	5 32	1030	B 0	0	0	0	– 8
71	9234/58	33	F	(+)	0	+	+	0	5 0	834	B 0	0	0	0	1 8
72	8286/60	20	F	0	0	0	0	0	5 32	1165	B 0	75 mill Ec.	0	0	2 8
73	7983/60	45	F	+	+	0	(+)	0	4 80	905	—	3 mill Ec.	0	0	–/7
74	1153 60	62	F	+	+	0	0	0	–	980	—	0	0	0	1 6
75	68 62	39	M	0	0	0	0	0	–	860	B 0	>100 000 Ec.	+	0	–/7
76	9232 61	63	F	+	+	0	+	0	4 67	870	B 0	>100 000 Ec.	0	0	2 8
77	6947 60	20	F	0	+	0	–	0	5 19	1120	B +	0	0	0	– 8
78	5539/64	43	M	0	0	0	0	0	—	415	B +	0	0	0	2 7
79	8697/61	41	F	+	0	0	0	0	5 2	1090	B 0	0	+	0	– 8
80	4234 61	26	F	+	+	0	+	0	4 88	1170	B 0	0	+	0	– 8

Cloudy ill smelling ur	Pregnancies (no.)	Phenacetin abuse	Previous bladder cath	BP (mm Hg)/24 hours or on admission	BP (mm Hg)/24 hours	Ocular fundi (Keith & Wagener)	Serum creatinine (mg/	Water loading test (mO mol 1 l)	Proteinuria	PAH	Inulin	Endog creatinine	General signs of di	Intercurrent disea
18	19	20	21	22	23	24	25	26	27	28	29	30	31	32
						GROUP A1 (continued)								
+	III	0	–	145 90	100 65	0	1 4	67	0	383		93	+	
+	IV	0	–	135 80	115 65	0	1 1	50	0	470	–	116	+	
		–	0	130 90	100 60	II-(III)	2.0	157	(+)	3?0	–	5[illegible]	+	B.l. re pap II.
+	I	0	–	170/100	120 85	0	4 0	140	+	89		–	+	Art. h
–	II	–	+	100 65	80 50	0	1.5	–	0	[illegible]65		50	–	B.l. re pap II
–	V	+	–	165/95	170 75	I	0 9	340	(+)		–	–	+	
+	II	–		140 95	120 60	0	1.3	265	0	285	–	61	+	
+	0	+	0	180 120	135/95	0	1 5	88	(+)	102	–	30	–	Art. h Pap II urin
	I	+	–	165/105	125 85	0	2 3	210	– +	215		47	+	Papill Art.
+	0	0	0	255/1?0	1?0 100	II	1 0	70	0	450	–	98	+	Art. 1
0		I	0	–	140 85	120 70	0	2 0	105	+	207		45	+
0	0	0	0	+	180/100	135 70	1	1 3	270	(+)	418	–	113	+
0	0	0	0	0	135 85	100 70	0	1 1	45	0	–		–	+
0	0	0	0	0	115/70	95 50	0	0 9	75	0	539	–	1[illegible]5	+
+	–		+	0	130 85	115/70	0	1 9	310	+			19	+
	–	III	0	0	150 80	115 65	I	[illegible]5	134	0			–	+
+	0	0	0	+	190 105	130 80	I	1 4	115	0	295	–	49	+
+	–	0	0	0	115 85	110 70	0	1 4	78	0	485		93	+
0	+	I	0	+	160 85	130 70	0	1 6	100	(+)	40		63	+
(+)		I	+	–	1 0/70	95 40	0	1 0	1[illegible]5	0		–	–	+
+		0			120 80	95 55	0	1 7	94	(+)	184	–	51	–
0	–	0	0	0	135 85	95 60	0	1 1	67	0	295		75	–
	–	0	0	+	120 90	100 65	0	1 3	57	0	3 4	–	8	+
0	0	0	–		115/75	105 65	I	6 8	200	+	37		9	+
0	–	1	0	+	1 5 85	105 60	0	2 6	87	0	173		43	–
0	( )	0	+	–	160 90	1[illegible]0 70	1	1 8	[illegible]20	(–)	155		35	+
0		0	+	0	130 80	95 50	0	1 ?	110	0	503	–	101	+
0		I	+	+	135 80	115/70	0	2 4	110	0	81	–	41	+
0	0	I	0	–	1?0 80	110 70		1 4	100	(+)	?99	–	84	
0	+	II		–	135 90	115 70	0	1 7	155	(+)	133		71	+
0	0	I	+	0	170 75	–	–	1 6	85	(+)	–	–	–	+
		0	+	0	155 100	125 80	1	2 4	80	0	30	–	59	+
0		VIII	0	+	150 95	1?0 65	0	0 8	85	(+)		–	–	+
0	0	II	0	+	115 45	1 5 85	0	4 6	240	–	–	–	–	+

Intercurrent disease column entries continued (truncated at page edge): "Art. 1" (beside rows 140 85 / 180/100); "Art. 1" (beside row 190 105); "Bil. r pap l" (beside row 120 80); "B.l. r pap l" (beside row 120 90); "Bil. r pap" (beside row 1 5 85); "B.l. pap" (beside row 160 90); "Art." (beside row 155 100).

GROUP A2 Acute and suspected chronic non-obstructive pyelonephritis (< 3 cardinal criteria)

18	19	20	21	22	23	24	25	26	27	28	29	30	31	32
0	( )	I	0	0	115 65	85 55	0	0 8	145	0	(717)	(163)	(87)	+
		0		–	115 70	105 55	0	1 4	50	0	–		–	+
0	0	I	0		120 65	95 50	0	0 9	60	(+)				+
0		II	0		125/90	110 65	0	1 0	60	(+)		–	(9 1)	+
0		0	(+)	+			–	0 7	80	0	564	–	108	+
0	0		0		120 80	110/70	0	0 9	–	(+)	635	–	153	+
											3 1	–	103	+
+		0	0	+	160 95	130 70	0	1 1	100	0	385	–	60	+
		I			130 85	90 65	0	1 1	73	0	386	–	90	–
0	0	–	+	0	10 90	175 80	1	4 4	3 8	+				

Table IVb *Cardinal criteria and salient data*

Case no	Case Record no	Age at invest (yrs)	Sex	Cardinal criteria of pyelonephritis: Cystitis without fever	Cystitis with fever	Chills without cystitis	Hist of loin pain	Loin tenderness to palpation	Acidif abil (pH)	Max conc ability (mOsmol/lit)	Histologic exam B = Biopsy R = Resection or ectomy A = Autopsy	*Bacteriuria* no /ml	Urinary sediment	Roentgenol exam	No pos criter a/No criteria tested
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
GROUP B1 Chronic pyelonephritis with signs of urinary tract obstruction (≥ 3 cardinal criteria)															
81	4978/58	51	F	+	0	0	0	0	5 6	445	B +	125 mill Ec	+	+ ×	7 8
82a	5824/63	47	F	+	+	–	+	+	7 1	331	B +	16 5 mill Ec.	+	+ ×	8 8
82b		48							–	–	–	900 000 Pm	+	–	
82c		49							6 00	385	–	>100 000 Pm	+	–	
82d		51							6 38	465	–	900 000 Pm	+	–	
83a	† 7681/59	42	F	+	+	0	+	+	–	–	–	>100 000 Ec Aa	+	+ ×	8 8
83b		42							6 0	345	–	66 mill Ec Aa	+	–	
83c		43							6 23	310	A +	167 mill Aa	+	–	
84a	7775/62	28	F	0	0	+	+	0	3 6	377	–	50 mill Ec	+	+ ×	6 7
84b		32							5 05	360	–	17 mill Ec	+	–	
85	8804/60	60	M	+	+	0	(+)	0	4 85	670	B +	>100 000 Ec.	+	+ ×	6 8
86a	1791/62	54	F	+	+	0	+	+	–	–	B +	>100 000 Pm	+	–	8 8
86b		56							5 15	425	–	140 mill Ec	+	+ ×	
86c		57							6 04	495	–	2 6 mill Pm	+	–	
87	5477/61	53	F	0	+	+	+	0	–	244	B +	>100 000 Ec	+	+ ×	6 7
88	3883/62	33	F	0	(+)	+	+	+	4 92	940	–	2 5 mill Ec	+	+ ×	5 7
89	1293/59	52	F	+	+	0	0	0	6 6	505	R +	150 000 Pv	+	+ ×	7 8
4c	7655/65	48	F	+	+	+	+	0	5 84	513	B +	700 000 Ec Pm	+	+ ×	7 8
90a	† 245 65	58	F	+	+	+	+	+	6 38	–	B +	>100 000 Pm	+	+ ×	7 7
90b		61							–	–	A +	>100 000 Pm	+	+ ×	
91	468/61	55	F	0	+	0	+	0	–	550	R +	0	0	+ ×	4 7
92	1870 63	43	F	0	+	0	+	0	–	575	B R +	3 mill Ec	+	+ ×	6 7
93	1627/59	61	F	0	+	+	+	0	5 5	685	–	2 mill Ec.	+	+ ×	6 7
94	8402/61	42	F	+	(+)	0	+	+	5 95	385	B +	7 mill Ec	+	+ ×	8 8
95	4234/62	51	F	+	0	0	0	0	5 77	477	–	0	+	+ ×	5 7
96	2600 61	22	F	0	+	0	+	+	5 15	1070	–	>100 000 Ec	+	0 ×	4 7
97	1104/61	37	F	0	0	0	0	0	6 2	575	R +	>100 000 Ec	–	+ ×	6 8
98	2208 61	44	F	+	+	0	(+)	0	5 40	980	B +	>100 000 Ec	–	+ ×	6 8
99a	6357/63	45	F	0	0	+	0	+	5 .0	540	B +	300 000 Pm	–	–	7 8
100	4107/60	16	F	0	+	0	+	+	5 40	1095	–	170 000 S aur	0	+ ×	5 7
101	3439/60	28	F	+	+	0	+	+	5 06	715	–	>100 000 Ec			6 7
102a	2648/61	52	F	0	+	0	(+)	0	5 50	485	–	230 000 Aa	0		5 7
103	9118 61	28	F	+	+	0	+	+	5 00	567	B –	>100 000 Ec	0		6 6
104	1607/62	37	F	0	0	+	+	0	5 69	480	B –	>100 000 Ec	0		6 8
105a	3686/60	38	F	0	0	+	(+)	0	5 35	459	–	250 000 Pm			6 7

Symbols
0 Nothing pathologic observed or answer to question negative
+ Pathologic finding or answer to question positive
(+) Doubtful positive finding or doubtful positive answer
× Pathologic finding without relevance to pyelonephritis
– Not investigated or questioned

Column 1
a b c and d denote examinations on different occasions in the same patient
Column 13
Ec *Escherichia coli* coliform organisms
Aa *Aerobacter aerogenes*
Pm Pv *Proteus mirabilis* *P vulgaris*
S aur *Staphylococcus aureus*
S alb *Staphylococcus albus*
Ent *Enterococci*

Haematuria	Cloudy ill-smelling urine	Pregnancies (no.)	Phenacetin abuse	Previous bladder catheteriz.	BP (mm Hg) 4 hours (max) or on admission	BP (mm Hg)/24 hours (min)	Ocular fundi (Keith & Wagener) F II	Serum creatinine (mg/100 ml)	Water loading test (mOsm l/l)	Proteinuria	Clearances ml min 1.73 m BSA: PAH	Inulin	Endog creatinine	General signs of disease	Intercurrent diseases
17	18	19	20	21	2	23	24	25	26	27	28	29	30	31	3?

OUP B1 *Chronic pyelonephritis with signs of urinary tract obstruction (≥ 3 cardinal criteria)*

–	(+)	0	0	0	130 75	110 60	–	1.8	228	(+)	(31)	(48)	(65)	0	Hydronephr R.
															Vesico-ureter reflux R.
0	(+)	1	0	0	150 85	100 60	0	2.0	129	(+)	(119)	(18)	(75)	+	Bl nephrol th
					130 70	110 60	–	2.4	–	(+)	169	–	48		
					–	–	0	2.3	215	(+)	(210)	(77)	(48)		
					160 90	115/70	I	1 8	151	(+)	184	–	47		
+	–	0	0	±	–	–	0	–	–	(+)	66	–	27	+	Bl nephrolith.
					150 85	110 70	0	3 1	266	(+)	(58)	(2.)	(31)		
					–	–	I	5 1	–	+	–	–	–		
0	+	I	0	–	140 100	1 5 80	0	1 7	203	++	(215)	(47)	(68)	+	Art. hypert.
					2 0 140	160 110	I II	3 4	205	++	(104)	(20)	(77)		Bl. renal papill. necr
															Bl. nephrolith.
0	0		0	+	165 90	135 80	I	1 7	155	±	12.	–	52	+	Hypertroph. prost.
0	–	I	+	(+)	140 85	125 70	I	–	–	(+)	–	–	–	+	Bl renal papill. necr
					160 85	125 65	I		145	(+)	161	–	36	+	Nephrol th L.
					175 90	130 65	I II	1 9	13.	0	(119)	(74)	(19)		
–	–	0	0	+	–	–	–	–	–	(+)	301	–	85	+	Nephrolith. R
0	–	IV	0		100 70	85 40	0	1 2	80	(+)	(4 5)	(75)	(7)	+	Nephrol th L.
0	+	I	0	–	265/140	200 105	II	1 3	120	(+)	377	(83)	143	+	Art. hypert.
															Nephrol th R
	0	I	0	–	135 80	–	0	1 6	84	(+)	(68)	(40)	(46)	0	Nephrolith. L.
–	–	I	–	+	115 65	100 50	–	4 3	180	(+)	30	(12)	40	+	Art. hypert
					200 105	150 85	II	3 1	–	++	(78)	–	(..)		Bl. nephrol th.
0	–	0	0	0	240 125	180 95	II	1 3	185	0	.33	–	58	+	Art. hypert.
															St. post perinephr L.
		J	0	∓	165 95	145 80	0	1 0	60	(±)	(509)	–	(81)	+	Bil. renal papill. necr
															Nephrol th. L.
	0	0	(±)	+	155 75	1.0 55	0	3 1	145	0	105	(19)	22	+	Nephrolith. L.
0	0	III	–		175 115	140 70	II	.1	155	(+)	219	–	87	+	Art. hypert.
															Bl. renal papill. necr
–	–	I	±	–	140 100	110 60	0	.1	170	0	.6	–	57	+	Art. hypert.
															Bl. renal papill necr
															Uretherol th. R.
0	–	0	0		175 85	95 60	–	0 9	65	0	–	–	–	+	Atony vesic. urin.
															Vesico-ureter reflux R.
0		I	–	+	180 120	140 100	I	1 3	–	(+)	297	–	101	0	Art. hypert.
															Nephrol th R
0	0	0	0	+	130 80	95 60	0	1.3	61	(+)	536	–	93	0	Neph ol th. R
0		0	0		140 95	115 75	0	1 9	90	0	171	–	44	∓	Nephrol th. R.
0		0	–	–	135 30	100 60	0	1 1	31	–	349	–	1 5	+	Vesico-ureter reflux R
	+	II	0		1 575	105 55	0	1.2	101	(+)	258	–	106	+	Bil. nephrol th.
															Uretherolith. R.
		0	+		210 1.0	140 90	I II	2 8	1 5	(+)	1.1	–	34	+	Art. hypert.
															Nephrol th. L.
0		0	0	0	1 575	100 60	0	1..	71	0	39	–	93	+	Hyperparath r
															Nephrol th L.
	–	II	0	–	190 75	105 60	0	2 0	1 8	0	78	–	71	+	Hydronephr L.?
0	0	I			145 80	170 70	0	2.1	150	0	333	–	65	+	Renal papill. necr R.
															Nephrol th. R

Col m 2 and)
When only one alue is g en for the blood pressure it denot th t on adm ssion. When a dia tolic pressure of ≥100 mm Hg w s recorded on any occa on it is noted n the table arterial hyperi sion irrespect of any f nd b gel.

Col mn 27
± ++ +++ denote slight, moderate and hea y proteinuria
C l mn 8–30
Bracketed alues denote that the test was made d rectly after ureteric catheterizat on.

Table IVb (*continued*)

Case no	Case Record no	Age at invest (yrs)	Sex	Cardinal criteria of pyelonephritis: Cystitis without fever	Cystitis with fever	Chills without cystitis	Hist of loin pain	Loin tenderness to palpation	Acidif abil (pH)	Max conc ability (mOsmol/kg)	Histologic exam B = Biopsy R = Resection or ectomy A = Autopsy	Bacteriuria no /ml	Urinary sediment	Roentgenol exam	No pos criteria/No crite a te ted
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
GROUP B2 Renal disease (except pyelonephritis) with unilateral or mainly asymmetric involvement															
106	4039/61	34	F	0	0	0	+	0	–	975	–	0	0	0 ×	1 6
107	3799/65	18	F	0	0	0	0	0	5 0	–	R +	0	0	0 ×	1/7
108	8562/59	36	F	0	0	+	+	0	4 91	535	B +	160 mill Ec	0	0	4 8
109	8695/60	52	M	0	0	–	–	0	–	–	–	0	0	0 ×	0 5
110	6381/61	27	M	–	–	–	–	0	–	–	–	50 000 Ec	+	0 ×	1 4
111	9950/58	62	M	0	0	0	0	0	5 05	310	R 0	0	0	+ ×	8
112	6149/59	61	M	0	0	0	0	0	–	–	R +	0	0	0 ×	1 6
113	7373/60	61	F	0	0	0	0	0	6 04	737	B +	6 mill Ec	+	+ ×	6 8
114	1738/63	55	F	+	0	0	+	0	6 23	930	–	600 000 S alb	+	– ×	5 7
115	5574/62	36	F	0	0	+	+	+	6 14	300	B 0 ×	20 mill Aa.	+	0 ×	6 8
116a	3227/61	17	F	0	0	0	+	0	–	–	B 0 ×	0	0	–	3 8
116b		18		0	+	0	0	+	4 84	1060	B +	0	0	0	
GROUP B3 Arterial hypertension with stenosis or aneurysm of the main renal artery															
117a	3532/62	55	F	+	0	0	0	(+)		–	–	0	0	0 ×	1/5
117b	3532/62	56	F							–	–	0	0	–	
118	3794/58	30	F	0	0	0	0	0	–	–	R 0 ×	0	0	0	0 6
119	1069/62	66	M	0	0	0	0	0	–	–	–	0	0	0 ×	0 5
120	7084/61	33	F	0	0	0	–	0	–	10?4	B 0 ×	0	+	0 ×	1/7
121	2765/60	52	M	0	0	0	0	0	–	1025	–	0	0	0 ×	0 6
122	† 8361/61	56	F	+	0	0	(+)	0	–	–	A 0	0	–	0 ×	. 6
123	6008/62	61	F	0	0	0	0	0	–	–		0	0	0 ×	0 5
124	1282/62	53	M	0	0	0	–	0		–	–	0	0	0 ×	0 5
125	671/62	68	M	0	0	0		0		–	–	0	0	0 ×	0 5
126	411/62	63	F	0	0	0	0	0			–	–	0	0 ×	0 4
127	9186/61	64	F	0	0	0	0	0	–	–		0	0	0 ×	0 5
128	5803/62	45	M	0	0	0	0	0	–	–	–	0	0	0 ×	0 5
129	6358/62	54	F	+	0	0	0	0		–		0	0	0 ×	1/5
130	501/63	49	F	0	0	0	0	0	–	–	–	0	0	0	0 5
131	3395/61	57	F	(+)	0	0	0	0	–	–		0	0	0	0 5
GROUP B4 Renal disease with expected homogeneous involvement of both kidneys															
132	2766/62	53	M	0	0	0	0	0	–	–		0	0	0	0 5
133	5865/62	31	F	+	+	0	0	0	–	–	–	0	0	0	1 5
134	† 1524/61	33	M	0	0	0	0	0	–	407	A 0 ×	0	0	0	1 7
135	2603 61	46	F	(+)	0	0	0	0	–	695	B 0 ×	~100 000 Pv	0	0	7
136	4667/58	49	M	0	0	0	(+)	0	5 2	584	B 0 ×	0	0	0	1 8
137	† 7143/58	23	M	0	0	0	0	0	–	745	A 0 ×			0	1 5
138 a	† 1198 60	33	F	0	0	0	+	0	–	565	B 0 ×	0	0		1 6
138 b		35							–		A 0 ×	0	0		
139	9432/59	35	F	+	+	0	+	0	5 4	–	B 0	0	0	0	7
140	2715/62	19	F	0	0	0	0	0			B 0 ×	0	0	0	0 6
141	2427/65	42	F	0	0	0	0	0	–	–	B 0 ×	0	0	0	0 6
142	7323/60	44	M	(+)	0	0	0	0	4 95	688	B 0 ×	0	0	0	1 8
143	† 4454 62	28	M	0	0	0	0	0			B A 0 ×	0	0	0	0 6
144	3703 60	14	F	0	0	0	0	0	5 20	835	B 0 ×	74 mill Aa	–	0	8
145	5058/64	39	F	0	0	+	+	0	–	318	B 0 ×	17 mill Ec	0		3 6
146	6307/65	31	M	0	0	0	0	0	–	5 0	B 0 ×	0	0		7
147	8963/60	35	F	0	0	0	0	0	5 4	360	R 0 ×	0	0		3 8
GROUP B5 Patients with symptoms from the lower urinary tract in whom involvement of the kidneys could not be ruled out															
148	8890 58	37	F	0	0	0	–	0	–		–	0	0	0	1 5
149	310 59	42	F	+	0	0	0	0	–	875	B 0	0	0	0	1 7
150	8825/60	41	F	+	0	0	+	0	4 9	8.5	B (–)	0	0	0	. 8
151	3971/60	20	F	+	0	0	0	0	–	1130	B 0	0	0	0	1 7
152	6418 60	32	F	+	+	0	0	0	5 10	1000	B 0	500 mill Ec	0	0	. 8

Case no	Haematuria	Cloudy ill smelling urine	Pregnancies (no)	Phenacetin abuse	Previous bladder catheteris	BP (mm Hg)/24 hours (mss) or on admission	BP (mm Hg) 4 hours (m n)	Ocular fundi (Keith & Wagener) FH	Serum creatinine (mg/100 ml)	Water loading test (mOsmol/lit)	Proteinuria	Clearances ml min/1 73 m BSA PAH	Inulin	Endog creatinine	General signs of disease	Intercurrent diseases	Diagnosis	Verification O = Oper H = Histol
1	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	3.	33	34
GROUP B2 Renal disease (except pyelonephritis) with unilateral or mainly asymmetric involvement																		
106	0	0	II	0	–	115 95	95 60	0	1 0	–	0	(608)	(118)	(104)	+		1	
107	0	0	0	0	0	200 120	150 100	1	1.2	–	0	–	–	–	+	Art. hypert.	2	
108	+	0	0	0	0	140 85	100 65	0	1 3	75	0	(390)	(57)	(80)	+		3	
109	–	–		–	–	175 10	–	I II	1 6	215	(+)	(274)	(79)	(70)	–	Art. hypert. Bil. nephrol th.	4	
110	+	0		0	0	140 80	–	–	1 3	–	(+)	–	–	(97)	+		3	
111	0	0			+	145/90	–	–	–	152	0	(531)	–		+		6	
112	0	0		0	–	190/100	155/75	II	1.2	–	+	(81)	(60)	(65)	+	Art hypert.	7	
113		0	0	0	–	210 110	155/90	I II	1 0	115	0	393	–	87	0	Art hypert.	8	
114		0	0	0	0	180/95	110/70	0	1 1	210	0	338	–	85	+		9	
115		+	I	0	0	115 80	105 60	0	1 2	140	(+)	3.6	–	83	+		10	H
116a	0	+	0	0	0	170 85	105 70	0	1 1	–	0	638	–	119	+		3	H
116b								0	1 1	–	+	46	–	105	–			
GROUP B3 Arterial hypertension with stenosis or aneurysm of the main renal artery																		
117a			III		–	215/135	145/70	I	1 4		+ + +	–	–	–	+		11	O
117b						220 115	180 105	I-(II)	1 3	–	0	–	–	–	–			O
118	0	0	I	0		240 150	200 130	III	1 4	–	–	(286)	(88)	(47)	+	Hypoplas. R. kidney	12	O
119					0	170/105	150 80	II	1.2	–	+		–	–	–		13	
170			0	–	+	270 140	150 80	II-(III)	1 0		(+)	–	–	–	–		12	
1 1					0	260 150	230 130	II	1 5	–	0	–	–	–	–		13	
1	0	0	I	0	0	220/105	170 100	II	1 1	–	(+)	303	–	76	+		12	O
1 3	0	0	III	0	+	210 100	140 65	II	0 8	–	0		–	–	0		1	
1 4				–	0	175/110	120 85	II	1 4		–	–	–	–	+		12	O
1 5					0	185/105	165 70	–	–		0		–	–	–		1	
1 6			II		–	200/100	160 85	0	1 0		0		–	–	+		14	
127			0		+	190 170	140 60	–	1 0	–	0		–	–	+		13	
1 8					0	2.5 130	180 100	II (III)	1 6	–	+ +	184	–	6	–		12	O
179			II		0	245/120	140 100	II	1 0		0		–	–	+		12	O
130	0	0	0	0		265 140	200 170	II III	0	–	0		–	38	+		12	O
131	0	(–)	0	0	0	240 145	205 100	III	1 0	–	(+)	2.4	–	67	+		1.	
GROUP B4 Renal disease with expected homogeneous involvement of both kidneys																		
13	0				0	200/120	155 100	0	1 4		0		–	–	0		15	
133			VII		–	140 110	125 90	I	1 0		0	–	–	–	+		15	
134	0	0				2.5 150	180 170	III	2.9		+	93	(33)	36	+		16	H
135	( )	0	0	0	+	200 175	160/100	I	0 9	70	0	417	–	83	+		15	H
136		0		–	+	160/95	125/75	0	1 2	217	+ +	887	–	84	+		17	H
137	0			–	–	140 100	–	0	1 4	–	–	(410)	(60)	(65)		Art. hypert.	17	H
138a	0	0	0	–	(+)	140 90	125 55	0	1 7	120	+	(150)	(21)	(1)	+	Art. hypert.	3	H
138b						220 140	180 115	IV	3..	–	+ +	–	–	–	–			
139		( )	III			130 80	105 60	0	1 2	65	(+)	(357)	(63)	(8)	+		3	
140			I	–		115 65	90 45	0	1 3	–	+ + +	(808)	–	(71)	–		3	H
141			0		+	145/95	130/75	0	1 8	–	+ +	–	–	–	–		17	H
14				0	0	190 140	145 75	II	1.2	158	+ + +	(563)	(113)	(107)	+	Art. hypert.	3	H
143	0	0		0	0	150/95	135 80	0	2.6	–	+ + +	491	–	104	–		17	H
144	0	0	0	0	0	140 80	105 55	0	1.2	160	+ +	537		94	0		17	H
145	0	0	IV	0	0	165 110	140 90	I-(II)	3 1	–	(+)	110	–	27	+	Art. hypert.	3	H
146	0	0			0	150 105	1.5 80	0	2.7	6	+	191	–	49	0	Art hypert.	18	H
147	0	0	0	+	0	130 90	110 70	0	1 9	160	(+)	2.68	–	66	+		18	H
GROUP B5 Patients with symptoms from the lower urinary tract in whom involvement of the kidneys could not be ruled out																		
148			II		–	135 75	90 45	0		–	(+)	(57?)	(17?)	(159)	+			
149	( )		II	0	0	115 70	95 55	0	1 4	145	0	366	(8?)	68	+			
150			IV			170 75	95 60	0	1 0	85	(+)	(6 4)	(88)	(110)	+			
151		0	1	0	0	120 75	90 60	0	0 8	90	0	–	–	–	0			
15		+	I	0	–	13 5	90 60	0	1 0	85	–	413		144	+		19	

Hypoplas. R kidne [?] Dyspl . L. kidney Chron glom. nephr art. Sten. R main renal art. Sten R. main renal art.
H perparathyr H droneph R. Nephrolith L. – Resection sten. main renal art. Ess. hypert. M lign. art. hypert.
R kidney – 56. [?] Hypoplas. L. kidney Renal TB L. Renal yndrom Chron. intersti. nephr [?] Urethrit Ne
TB R Susp. renal t...dosus. Bil. aneurysm main renal

Table IVb (*continued*)

Case no	Case Record no	Age at invest (yrs)	Sex	Cardinal criteria of pyelonephritis: Cystitis without fever	Cystitis with fever	Chills without cystitis	Hist of loin pain	Loin tenderness to palpation	Acidif abl (pH)	Max conc ability (mOsmol/lit)	Histologic exam B = Biopsy R = Resection or ectomy A = Autopsy	Bacteriuria no/ml	Urinary sediment	Roentgenol exam	No pos criteria/No criteria tested
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
GROUP B2 Renal disease (except pyelonephritis) with unilateral or mainly asymmetric involvement															
106	4039 61	34	F	0	0	0	+	0	–	975	–	0	0	0 ×	1 6
107	3799 65	18	F	0	0	0	0	0	5 0	–	R +	0	0	0 ×	1 7
108	8562/59	36	F	0	0	+	+	0	4 91	535	B +	160 mill Ec	0	0	4 8
109	8695/60	52	M	0	0	–	–	0	–	–	–	0	0	0 ×	0 5
110	6381/61	27	M	–	–	–	–	0	–	–	–	50 000 Ec	+	0 ×	1 4
111	9950/58	62	M	0	0	0	0	0	5 05	310	R 0	0	0	+ ×	– 8
112	6149/59	61	M	0	0	0	0	0	–	–	R +	0	0	0 ×	1 6
113	7373/60	61	F	0	0	0	0	0	6 04	737	B +	6 mill Ec	+	+ ×	6 8
114	1738/63	55	F	+	0	0	+	0	6 23	930	–	600 000 S alb	+	+ ×	5 7
115	557⌞ 62	36	F	0	0	+	+	+	6 14	300	B 0 ×	20 mill Aa	+	0 ×	6 8
116a	3227/61	17	F	0	0	0	+	0	–	–	B 0 ×	0	0	–	
116b		18		0	+	0	0	+	4 84	1060	B +	0	0	0	3 8
GROUP B3 Arterial hypertension with stenosis or aneurysm of the main renal artery															
117a	353⌞/62	55	F	+	0	0	0	(+)	–	–		0	0	0 ×	1 5
117b	3532/62	56	F						–	–	–	0	0		
118	3794/58	30	F	0	0	0	0	0	–	–	R 0 ×	0	0	0 ×	0 6
119	1069 62	66	M	0	0	0	0	0		–		0	0	0 ×	0 5
120	7084/61	33	F	0	0	0		0	–	1074	B 0 ×	0	+	0 ×	1 7
121	2765/60	52	M	0	0	0	0	0	–	1025		0	0	0 ×	0 6
122	† 8361/61	56	F	+	0	0	(+)	0	–	–	A 0	0	+	0 ×	6
123	6008/62	61	F	0	0	0	0	0	–	–	–	0	0	0 ×	0 5
124	1282/62	53	M	0	0	0		0				0	0	0 ×	0 5
125	671/62	68	M	0	0	0		0	–	–		0	0	0 ×	0 5
126	411/62	63	F	0	0	0	0	0	–	–			0	0	0 4
127	9186/61	64	F	0	0	0	0	0	–	–	–	0	0	0 ×	0 5
128	5803 62	45	M	0	0	0	0	0				0	0	0 ×	0 5
129	6358/62	54	F	+	0	0	0	0		–	–	0	0	0 ×	1 5
130	501/63	49	F	0	0	0	0	0	–	–		0	0	0	0 5
131	3395/61	57	F	(+)	0	0	0	0				0	0	0	0 5
GROUP B4 Renal disease with expected homogeneous involvement of both kidneys															
132	2766/62	53	M	0	0	0	0	0			–	0	0	0	0 5
133	5865 62	31	F	+	+	0	0	0	–	–		0	0	0	1 5
134	† 1524 61	33	M	0	0	0	0	0		407	A 0	0	0	0	1 7
135	2603 61	46	F	(+)	0	0	0	0	–	695	B 0 ×	~100 000 Pv	0	0	– 7
136	4667 58	48	M	0	0	0	(+)	0	5 2	584	B 0 ×	0	0	0	1 8
137	† 7143 58	23	M	0	0	0	0	0		745	A 0 ×			0	1 5
138 a	† 1198 60	33	F	0	0	0	+	0	–	565	B 0 ×	0	0		1 6
138 b		35								–	A 0 ×	0	0		
139	9432/59	35	F	+	+	0	+	0	5 4		B 0	0	0	0	7
140	2715 62	19	F	0	0	0	0	0		–	B 0	0	0	0	0 6
141	24 7/65	42	F	0	0	0	0	0	–		B 0 ×	0	0	0	0 6
142	73⌞3 60	44	M	(+)	0	0	0	0	4 95	688	B 0 ×	0	0	0	1 8
143	† 4454 62	28	M	0	0	0	0	0			B A 0 ×	0	0	0	0 6
144	3703/60	14	F	0	0	0	0	0	5 0	835	B 0 ×	4 mill Aa		0	8
145	5058 64	39	F	0	0	+	+	0		318	B 0 ×	17 mill Ec	0		3 6
146	6307/65	31	M	0	0	0	0	0	–	5⌞0	B 0	0	0		7
147	8963 60	35	F	0	0	0	0	0	5 4	380	B 0 ×	0	0		3 8
GROUP B5 Patients with symptoms from the lower urinary tract in whom involvement of the kidneys could not be ruled out															
148	8890 58	37	F	0	0	0	+	0				0	0	0	1 5
149	310 59	42	F	–	0	0	0	0		875	B 0	0	0	0	1 7
150	8825 60	41	F	+	0	0	–	0	4 9	8⌞5	B ( )	0	0	0	2 8
151	3971/60	20	F	+	0	0	0	0		1130	B 0	0	0	0	(7
152	6418/60	32	F	+	+	0	0	0	5 10	1000	B 0	500 mill Ec	0	0	8

Table V Comparison between clinical and histologic observations in 82 cases of pyelonephritis (chronic or acute uncomplicated or complicated groups A1 A2 B1)

	Total no of cases			Agreeing clinical diagnosis						Doubtful		Not agreeing clinical diagnosis				
Group	A1	A2	B1	A1		A2		B1		A1		A1		A2		B1
	No	No	No	No	%	No	%	No	%	No	%	No	%	No	%	No
Specimen obtained at needle biopsy	52	9	12	34	65	2	22	12	100	2	4	16	31	7	78	
Specimen obtained at surgery or autopsy	4	–	5	4	100	–	–	5	100	–	–	–	–	–	–	–
Sum	82			57 = 70 %						2 = 2 %		23 = 28 %				

noting it as interstitial nephritis Moreover he pointed out that the criteria of Weiss & Parker (267) — periglomerular fibrosis, interstitial cell infiltration thyroid like tissue — are not pathognomonic to chronic pyelonephritis These features are also seen in patients with *e g* renal disease due to vascular lesions or with renal damage caused by toxic or other exogenous agents (*e g* phenacetin abuse)

Thus it is not possible to set up either clinical or histologic criteria which must always be fulfilled for a diagnosis of chronic pyelonephritis In the present study I have nevertheless included the histologic examination in the group of cardinal criteria since the finding of interstitial nephritis at this examination does when other criteria are fulfilled strengthen the suspicions of chronic pyelonephritis

The value of the histologic examination for the diagnosis is illustrated in Table V It shows that in about 70 per cent of the 82 cases of chronic or acute pyelonephritis — of both uncomplicated and complicated type — in which this examination was made the histologic features largely corresponded to the clinical symptoms and signs (at least three cardinal criteria) In 2 per cent the examiner was doubtful and in 28 per cent the microscopic features did not correspond to the clinical features Similar conditions were reported by Brod (29)

Brun & Raascsou (38 39) found no other clinical or histologic differences between patients who had consumed phenacetin and those who had not than the presence in some of the former cases of necrotic tissue in the biopsy specimen or a necrotic papilla in the urine Differences may also be observed at autopsy This agrees with my experience However Ringertz (211) like Spühler & Zollinger (239) stated that there are additional histologic lesions which distinguish chronic interstitial nephritis — such as occurs in abuse of phenacetin — from chronic pyelonephritis originating in the renal pelvis For further discussion of this question, reference is made to Hultengren (110)

6 *Bacteriuria*

Since pyelonephritis presupposes the existence of renal infection demonstration of bacteriuria was naturally of the greatest im

series of patients with chronic pyelonephritis that 45 per cent complained of dysuria and pollakiuria

2 *Tenderness to palpation*

On examination of the patients, particular attention was focused on the existence of tenderness to palpation over one or both loins Although this may occur in the presence of, *e g* polycystic kidneys or a renal tumour, it strongly supported the diagnosis when chronic pyelonephritis was suspected Brod (29) stated that in those who complained of pain in the loins (64 per cent of the material) tenderness to palpation was present

3 *Ammonium chloride load*

Decreased ability to acidify the urine is an important diagnostic sign in chronic pyelonephritis (77, 135) and in other renal diseases (10b, 272, 273)

The technique used in the ammonium chloride load test is described in Chapter VII It was not performed in patients with a tendency to acidosis nor in those with urea splitting bacteria in the urine The limit of normal renal acidifying ability was given by Bengtsson (10b) as pH 5 2, and by Wrong (272 273) as pH 5 5 I took pH 5 3 (18) as the normal limit on the basis of 11 determinations in 9 healthy subjects aged 23—54 years

4 *Renal concentration ability*

This was determined in mOsmol/lit The test was made after the patient had been under observation in hospital for a few days and had been given a standard diet

Like Bengtsson (10b) I set the limit of normal osmolarity at 800 mOsmol/lit irrespective of age, even though it has been shown that the osmolarity of the urine starts to decrease as early as 30—40 years of age (151, 264) The performance of the test is described in Chapter VII

5 *Histologic changes*

Renal biopsy in which one is fortunate enough to obtain a specimen of tissue with pyelonephritic lesions is, naturally, of great value for the diagnosis A biopsy specimen is often difficult to judge, particularly if it consists only of medullary tissue and does not contain any glomerulus Obviously the results were more reliable when the microscopic examination was made on a specimen of resected tissue, or post mortem The nature of the specimen on which the histologic diagnosis was based is shown in Table IV In the present study, it was of the utmost value that all the specimens were examined by the same pathologist Even if a biopsy specimen did not exhibit interstitial lesions this was not considered to argue against the diagnosis, since the specimen might have been taken from intact renal tissue, which is often present between the focal lesions

The histologic criteria of pyelonephritis were those set up by Brun & Raaschou (39) *i e*,

Cell casts
Interstitial cell infiltration
Invasive glomerulitis
Periglomerular fibrosis
Tubular atrophy
Peritubular fibrosis
Dilated tubules with acellular casts (thyroid like)

Brun (36b) has however stated that in recent years he has not made a diagnosis of chronic pyelonephritis on a histologic specimen but has confined himself to de

Table V *Comparison between clinical and histologic observations in 82 cases of pyelonephritis (chronic or acute uncomplicated or complicated groups A1 A2 B1)*

Group	Total no of cases			Agreeing clinical diagnosis						Doubtful		Not agreeing clinical diagnosis					
	A1	A2	B1	A1		A2		B1		A1		A1		A2		B1	
	No	No	No	No	%	No	%	No	%	No	%	No	%	No	%	No	%
Specimen obtained at needle biopsy	52	9	12	34	65	2	22	12	100	2	4	16	31	7	78		
Specimen obtained at surgery or autopsy	4		5	4	100	–		5	100						–	–	
Sum	82			57 = 70 %						2 = 2 %		23	28 %				

noting it as interstitial nephritis Moreover he pointed out that the criteria of Weiss & Parker (267) — periglomerular fibrosis interstitial cell infiltration thyroid like tissue — are not pathognomonic to chronic pyelonephritis These *features are also seen in patients with e g renal disease due to vascular lesions or with renal damage caused by toxic or other exogenous agents (e g phenacetin abuse)*

Thus it is not possible to set up either clinical or histologic criteria which must always be fulfilled for a diagnosis of chronic pyelonephritis In the present study I have nevertheless included the histologic examination in the *group of cardinal criteria* since the finding of interstitial nephritis at this examination does when other criteria are fulfilled strengthen the suspicions of chronic pyelonephritis

The value of the histologic examination to the diagnosis is illustrated in Table V It follows that in about 70 per cent of the 82 cases of chronic or acute pyelonephritis of both uncomplicated and complicated type — in which this examination was made the histologic features largely corresponded to the clinical symptoms and signs (at least three cardinal criteria) In 2 per cent the examiner was doubtful and in 28 per cent the microscopic features did not correspond to the clinical features Similar conditions *were reported by Brod (29)*

Brun & Raaschou (38 39) found no other clinical or histologic differences between patients who had consumed *phenacetin* and those who had not than the presence in some of the former cases of necrotic tissue in the biopsy specimen or a necrotic papilla in the urine Differences may also be observed at autopsy This agrees with my experience However Ringertz (211) like Spuhler & Zollinger (239) stated that there are additional histologic lesions which distinguish chronic interstitial nephritis — such as occurs in abuse of phenacetin from chronic pyelonephritis originating in the renal pelvis For further discussion of this question, reference is made to Hultengren (110)

6 Bacteriuria

Since pyelonephritis presupposes the *existence of renal infection* demonstration of bacteriuria was naturally of the greatest im

Table VIa *Survey of the cardinal diagnostic criteria in chronic non-obstructive pyelonephritis (grou*

Criterion	No of invest	Limit	Positive		Negative		D
			No	%	No	%	No
Cystitis without fever	66		33	50	33	50	
Cystitis with fever	68		46	68	20	29	2
Chills without cystitis	63		15	24	47	75	1
Hist of loin pain	69		48	70	17	25	4
Loin tenderness to palpation	69		29	42	40	58	
Acidif abil (pH)	60	pH 5 3	30	50	30	50	
Max conc ability (mOsmol/lit)	66	800 mOsmol/lit	57	86	9	14	
Histologic exam (*cf* Table V)	56		38	68	16	29	2
Bacteriuria (no /ml)	69	*100 000* bact/ml	63	91	6	9	
Urinary sediment	69	10 WBC/high power field	60	87	9	13	
Roentgenol exam	68		46	68	22	32	

portance This applied despite the fact that the urine may be sterile even in unquestionable pyelonephritis, as a result either of a non functioning kidney or of anti infective therapy

A thorough bacteriologic investigation was made in all the patients in the present series It included quantitative determination of the *bacteria, and determination of the sensitivity* to all the common chemotherapeutics and antibiotics (see Chap VII) When *e g*, *Staphylococcus albus* and diphtheroids were present separately or together on a few occasions, and then only in small numbers they were regarded as apathogenic and were not counted as a positive diagnostic criterion

A bacterial count was made and the value obtained was taken into account irrespective of whether or not the bacteriuria had given rise to symptoms *Asymptomatic* bacteriuria is considered to occur in about 4 per cent of healthy individuals who have not been hospitalized (128—130) and in up to 30 per cent of *patients* with severe intestinal diseases (147) and women with diabetes mellitus (127 257)

In accordance with Kass (128) I took 100,000 bacteria/ml as the lower limit of significant bacteriuria in specimens from the urinary bladder The corresponding figure for urine obtained by ureteric catheterization was 10,000 bacteria/ml (127) In the few cases where no bacterial count was made, bacteriuria was denoted as copious (corresponding to > 100 000 bact/ml) moderate *(corresponding to 10,000—100,000/ml)* or sparse (100—10,000/ml)

7 Urinary sediment

Ten or more leukocytes per high power field in the spun sediment were defined as definite pyuria In many cases, I was able to confirm that the sediment may be negative in patients with pyelonephritis despite bacteriuria I considered the most important findings in the sediment in pyelonephritis to be pyuria and the discrepancy between the numerous leukocytes and the few red cells Granulated cylinders were often present consisting of degenerated white blood cells and tubular epithelium *as a sign of involvement of renal tissue* (206) A feature regarded as especially important for the diagnosis was the demon

Table VIb *Survey of the cardinal diagnostic criteria in suspected chronic or acute non-obstructive pyelonephritis (group A2)*

Criterion	No of invest	Limit	Positive No	Positive %	Negative No	Negative %	Doubtful No	Doubtful %
Cystitis without fever	11		6	55	4	36	1	9
Cystitis with fever	11		5	45	6	55		
Chills without cystitis	11		1	9	10	91		
Hist of loin pain	10		4	40	5	50	1	10
Loin tenderness to palpation	11				11	100		
Acid abil (pH)	8	pH 5 3	2	25	6	75		
Max conc ability (mOsmol/lit)	11	800 mOsmol/lit	1	9	10	91		
Histologic exam (cf Table V)	9		2	22	7	78		
Bacteriuria (no /ml)	11	100 000 bact/ml	4	36	7	64		
Urinary sediment	11	10 WBC/high power field	3	27	8	73		
Roentgenol exam	11				11	100		

stration by Sternheimer & Malbin staining (243) of the characteristic vacuolated cells (85) Renal epithelium was often present

The aforementioned features as well as demonstration of bacteria in centrifuged specimens were regarded as positive findings

8 *Roentgenologic changes*

Excretory pyelography Dejdar (62) and Dejdar & Prat (63) stressed the value of roentgenologic examination of the kidneys in chronic pyelonephritis partly to demonstrate any asymmetric involvement (cf Chap 1) In the present series the roentgenologic diagnosis of chronic pyelonephritis was based on the criteria of Olsson (182) and that of renal papillary necrosis on the criteria of Lindvall (153)

Retrograde pyelography was only occasionally performed It was done either because renal function was greatly impaired or because one kidney was silent for some other reason

Selective renal angiography was performed in hypertensive patients with chronic pyelonephritis when arterial hypertension was suspected to be caused by stenosis of the main renal artery It was also done in the absence of hypertension when inflammatory foci or scarring were suspected

Urethrocystography On technical grounds this was carried out in only a few patients

A survey of the cardinal diagnostic criteria and the number and incidence of cases of chronic and acute non obstructive pyelonephritis in which they were fulfilled is given in Table VI together with the total number of investigations

B General Symptoms of Renal Disease

Patient s statements

The patient s statements about his complaints are naturally of great importance for the diagnosis This applies irrespective of whether or not he ascribes them to renal disease Obviously when such symptoms were mentioned or discussed due reservations had to be made for the unreliability of the patient s statements

Table VIa *Survey of the cardinal diagnostic criteria in chronic non-obstructive pyelonephritis (group A*

Criterion	No of invest	Limit	Positive		Negative		Doub
			No	%	No	%	No
Cystitis without fever	66		33	50	33	50	
Cystitis with fever	68		46	68	20	29	2
Chills without cystitis	63		15	24	47	75	1
Hist of loin pain	69		48	70	17	25	4
Loin tenderness to palpation	69		29	42	40	58	
Acidif abil (pH)	60	pH 5 3	30	50	30	50	
Max conc ability (mOsmol/lit)	66	800 mOsmol/lit	57	86	9	14	
Histologic exam (*cf* Table V)	56		38	68	16	29	2
Bacteriuria (no /ml)	69	100 000 bact/ml	63	91	6	9	
Urinary sediment	69	10 WBC/high power field	60	87	9	13	
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Table VIb *Survey of the cardinal diagnostic criteria in suspected chronic or acute non-obstructive pyelonephritis (group A2)*

Criterion	No of invest	Limit	Positive		Negative		Doubtful	
			No	%	No	%	No	%
Cystitis without fever	11		6	55	4	36	1	9
Cystitis with fever	11		5	45	6	55		
Chills without cystitis	11		1	9	10	91		
Hist of loin pain	10		4	40	5	50	1	10
Loin tenderness to palpation	11				11	100		
Acidif abil (pH)	8	pH 5 3	2	25	6	75		
Max conc ability (mOsmol/lit)	11	800 mOsmol/lit	1	9	10	91		
Histolog c exam (cf Table V)	9		2	22	7	78		
Bacteriuria (no /ml)	11	100 000 bact/ml	4	36	7	64		
Urinary sediment	11	10 WBC/high power field	3	27	8	73		
Roentgenol exam.	11				11	100		

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Reasons for consultation

All patients investigated and treated at the Renal Clinic, S t Eriks Sjukhus, either as out patients or in patients, were referred by other physicians, who had treated them for a varying period For this reason it was sometimes difficult to reconstruct the cause of their originally having consulted a physician However, whenever possible, the first symptom was noted

Duration of complaints

It was often hard to obtain any exact idea of the duration of the urinary tract symptoms This was due partly to the chronicity of the disease with alternating remissions and exacerbations Another contributory factor was that the onset was frequently insidious or completely asymptomatic In some patients, it took place already in childhood This was important information but was sometimes difficult to elicit In view of the difficulty of determining the duration of the symptoms in the individual case this information has been omitted from Table IV

Haematuria

Even though macroscopic haematuria is more common in other forms of renal disease it may occur in chronic pyelonephritis and be noticed by the patient As a rule, it is to be ascribed to passage of a concretion or a renal papilla, or to a mucosal lesion which, in turn is caused by infection

Appearance and smell of urine

Cloudy urine with a bad smell is obviously an important symptom which may lead to suspicions of urinary tract disease The patient not infrequently sought medical advice for this reason, and investigation — including microscopic examination of the sediment, bacterial culture and renal function tests — sometimes disclosed the existence of an otherwise asymptomatic chronic infection, possibly pyelonephritis Often, the patient noticed that passage of such urine was combined with back pain and possibly with a rise in temperature A history of such symptoms naturally strengthened the suspicions of chronic pyelonephritis

Influence of pregnancy

Attacks of acute pyelonephritis are known to be more frequent in pregnant women than in others Moreover, numerous investigations have shown that asymptomatic bacteriuria often occurs in pregnancy (49, 78 103 124, 125 128, 129 133 146 174, 190, 254) and that the incidence is higher than in non pregnant women It is also important to bear in mind that asymptomatic bacteriuria in pregnancy leads to clinically demonstrable pyelonephritis in 35—40 per cent of cases (129)

I considered it of interest to ascertain whether the patient s bladder had been emptied by catheterization in connexion with childbirth This was because such a procedure may cause infection of the lower urinary tract and thereby gradually lead to pyelonephritis The number of childbirths is therefore listed in Table IV as well as the minimum number of associated catheterizations of the bladder

Phenacetin abuse

It was extremely difficult to obtain any exact information about the amount of phenacetin (acetophenetidine) taken The patients adopted an evasive attitude when questioned on this matter They not infrequently underestimated their real consump

tion by conscious forgetfulness In view of the unreliability of their statements I did not wish to apply Schweingruber's (225) limit for abuse i e at least 1 g of phenacetin per day for more than 1 year or Bengtsson's (10b) lower limit of at least 0 3 g/day for at least 1 year Instead I denoted as abuse a regular preferably daily intake of phenacetin containing preparations for a long time (at least 1 year)

Most authors now agree that a relation exists between phenacetin abuse and renal papillary necrosis (10b 11 88 91 177 239) whereas others contend that additional substances — e g acetylsalicylic acid — may be able to cause interstitial nephritis (244) I shall not enter into a detailed discussion of the relation between phenacetin abuse and chronic pyelonephritis but refer to the abundant literature on the subject

In the present series the coexistence of chronic pyelonephritis and renal papillary necrosis is recorded in the tables but no attempt was made to distinguish between these cases and pure pyelonephritis as regards renal function This is because papillary necrosis without concomitant urinary tract infection and signs of chronic pyelonephritis was demonstrable in only two patients (cases 146 147)

Previous instrumentation

Since introduction of an instrument into the urinary tract may produce infection the patients were always questioned on this matter

C. General Signs of Renal Disease

Physical examination

The physical findings characteristic of *uraemia* and *inaemia* are too well known to require any description

Blood pressure and fundus changes

It cannot be ruled out that arterial hypertension may cause secondary more severe renal damage and thus mask any inappreciable asymmetric involvement (*cf* Chap X p 105) For this reason the arterial blood pressure is noted in the tables

For the same reason the optic fundi were inspected and any changes observed were divided into groups according to the classification of Keith & Wagener (*cf* Chap VII) Ophthalmoscopy was performed by an ophthalmologist

Serum creatinine

The serum creatinine was recorded as a test of renal function this determination is made routinely at the Central Laboratory of our hospital The test is comparable with the determination of BUN and NPN (118)

Water loading test of Volhard

This test was made in every patient Individuals with healthy kidneys can as a rule dilute the urine to an osmolarity of 50 mOsmol/lit or even lower However as far as I have been able to ascertain no investigation has been made to determine the dilution that is to be required if the value is not to be regarded as a sign of reduced renal dilution ability In our experience at the Renal Clinic this test is of little clinical worth in the diagnosis of renal diseases

Proteinuria

In chronic pyelonephritis protein is not usually present in the urine in any great quantity i e seldom in excess of 5 g/24 hours (29) I included this test because heavy proteinuria is more characteristic of other renal diseases than of pyelonephritis As far as possible I tried to determine the 24 hour excretion

It cannot be ruled out that, in a few cases, small quantities of vaginal secretion contributed to a positive protein reaction This source of error was generally eliminated by the technique of taking the sample with a glass tube (*cf* Chap VII)

Creatinine clearance

The *endogenous creatinine clearance* was determined in almost every case

Inulin clearance

On practical grounds the inulin clearance was determined only in those patients who underwent renal vein catheterization In the other cases, the endogenous creatinine clearance was determined instead, even if this is known to be an unreliable measure of the glomerular filtration rate (232)

PAH clearance

Since most of the patients in my series had decreased PAH extraction as a sign of renal damage, the PAH clearance is of little value as an expression of the renal blood flow The values were nevertheless included, to illustrate the use of this parameter as a diagnostic test in chronic pyelonephritis

D General Signs of Disease

This group includes such vague unspecific *symptoms as fatigue*, headache weight loss and dyspnoea but no urinary tract symptoms *Instead* the gastrointestinal symptoms dominate, *e g* nausea and sometimes vomiting and other digestive or intestinal complaints

Brod (28) found such unspecific symptoms in ¼ to ⅓ of his patients, and similar figures have been reported by other workers (135) The patients in my series were questioned about such symptoms and examined for their presence but they were not analyzed in any detail This is because as already mentioned, they are not significant either for renal disease in general or for chronic pyelonephritis in particular Such symptoms are common, but did not occur consistently in my cases In Table IV, + denotes their occurrance, and 0 that they were absent

In every case the ECG was recorded and a roentgenologic examination was made of the heart and lungs In addition the following determinations were made haemoglobin in blood red cell count, leukocyte count and differential leukocyte count, ESR serum chlorides, sodium potassium phosphorus calcium and alkali reserve The results of these investigations are given and commented on only when there is special reason to do so

The diagnostic observations listed in Table IV include *the finding of any complicating* disease of the kidneys and or lower *urinary tract as well as other complicating* diseases which might have influenced the *results of the tests* Particular attention was focused on diabetes mellitus since a relation has been stated to exist between this disease and chronic pyelonephritis (127 257) My series contained only one diabetic patent (case 30)

CHAPTER IV

DIAGNOSIS OF OTHER RENAL DISEASES

In addition to the cases of chronic pyelonephritis the present series contains some patients with other renal diseases The chief reason for including them was that they were regarded to be suitable as comparative material Since the diagnosis seldom presented any difficulties in these cases a discussion of the diagnostic criteria is superfluous All the investigations made in the diagnosis of chronic pyelonephritis were used in these cases also Consequently only brief mention will be made of the mo t important investigations apart from the fundamental ones

In *arterial hypertension* in which stenosis of the main renal artery was suspected renal angiography was needed to verify the diagnosis The urinary catecholamines were determined in all hypertensive patients to rule out the existence of phaeochromocytoma

In *chronic glomerulonephritis* and the *nephrotic syndrome* the customary urine and renal function tests were complemented by determination ot the total serum protein electrophoresis of serum proteins and the antistreptolysin and antistaphylococcal titres in serum

In *polycystic kidney* various roentgenologic methods we e used as diagnostic aids

Suspicions of *renal amyloidosis* could be verified in some cases by examination of a renal biopsy specimen

In two cases of *renal tuberculosis* with complicating chronic pyelonephritis a definite diagnosis was reached with the help of culture in Löwenstein medium and guinea pig inoculation

When *residual urine* was suspected to be p esent this was determined in the customary way

If *hyperparathyroidism* was suspected repeated determinations were made of the calcium and phosphorus in serum and urine This was complemented by roentgenologic examinations with the object of demonstrating the characteristic periosteal erosions and bone cysts

It need scarcely be pointed out that all the methods of examination described do not form part of the routine battery of tests used for d agnosis at the Renal Clinic All of them are unquestionably of diagnostic value in certain doubtful cases but in the majority of cases of renal disease the diagnosis can be established with satisfactory certainty without having to resort to such methods as *e g* renal vein catheterization and ureteric cathe erization This question is discussed in d tail in Chapter X (p 112)

CHAPTER V

CASE MATERIAL

The case material on which the present investigation is based consists of totally 152 patients investigated and treated at the Central Clinical Laboratory and the Renal Clinic in 1958—1963 inclusively All the patients had been hospitalized at least once for investigation and, at the time of writing, most of them are still under regular control in the out patient department These patients comprise merely a small proportion of all those treated at the Renal Clinic during the relevant period, only those who underwent bilateral renal vein and/or ureteric catheterization being included in the material

Healthy volunteers were used as controls for determination of the PAH extraction by the right and the left kidney (see Table III) They consisted of 11 subjects (8 men and 3 women) aged from 18—27 years (average 20 years)

In certain cases, the basis of selection was that the diagnosis of chronic pyelonephritis was somewhat doubtful In many patients who fulfilled several of the cardinal criteria of chronic pyelonephritis, the object was to ascertain whether or not involvement of the kidneys was asymmetric by means of a comparison between them with respect to such factors as the various parameters of function and the findings at roentgenologic examination In addition, renal vein and ureteric catheterization were performed in a few patients with other renal diseases than chronic pyelonephritis to demonstrate whether involvement was symmetric or asymmetric

For technical reasons, not all the patients in the present series could be investigated regarding each of the eight cardinal criteria listed in Chapter III The results of investigation nevertheless sufficed to classify them in groups by the clinical diagnosis, on the grounds described in the following The results of the investigations on which this classification was based are given in Table IV This table also shows how many patients in each of the groups were investigated with respect to all eight cardinal criteria and how many were investigated with respect to a limited number of them The number of patients in whom the relevant number of criteria were fulfilled can also be inferred from Table IV

Since my primary aim was to investigate the symmetry (asymmetry) of renal function the patients were divided into a number of groups according to the diagnostic symptoms and signs described in Chapters III and IV

— Cases in which it was considered doubtful whether symmetric or asymmetric renal function could be expected i e chiefly chronic and acute non obstructive pyelonephritis (groups A1 and A2)

— Cases of renal disease in which it was evident that the two kidneys were not damaged to the same extent e g chronic pyelonephritis with unilateral urinary tract obstruction such as a concretion or ureteric stricture as well as cases of e g arterial hypertension with stenosis of a renal artery unilateral renal tuberculosis and unilateral hydronephrosis (groups B1 B2 and B3)

— Cases in which the damage could be expected to be of more homogeneous type and to affect both kidneys to about the same extent i e chronic glomerulonephritis the nephrotic syndrome essential hypertension and cases in which no evident renal damage was demonstrable (groups B4 and B5)

Classification of Patients

Group A1 Chronic non obstructive pyelonephritis fulfilling three or more cardinal criteria (denoted in the figures by ●) 4 men and 65 women aged from 18—67 years (average 44 years)

Group A2 Suspected chronic non obstructive pyelonephritis fulfilling less than three cardinal criteria, as well as acute and recurrent acute non-obstructive pyelonephritis (◊) 2 men and 9 women aged from 20—63 years (average 39 years)

Group B1 Chronic pyelonephritis fulfilling three or more cardinal criteria and with signs of urinary tract obstruction (o) 1 man and 25 women aged from 16—61 years (average 43 years)

Group B2 Renal disease except pyelonephritis with unilateral or mainly asymmetric involvement (X) 4 men and 7 women aged from 17—62 years (average 42 years)

Group B3 Arterial hypertension with stenosis or aneurysm of the main renal artery (■) 5 men and 10 women aged from 30—69 years (average 54 years)

Group B4 Renal disease which could be expected to involve both kidneys more homogeneously and to about the same extent The nephrotic syndrome and chronic glomerulonephritis are denoted by ▲ renal papillary necrosis without signs of urinary tract infection by △ and essential hypertension by □ 7 men and 9 women aged from 14—53 years (average 35 years)

Group B5 Patients with symptoms from the urinary bladder and urethra, in whom involvement of the kidneys could not be ruled out (∧) 5 women aged from 20—42 years (average 34 years)

For reasons of space all cases belonging to groups B1—B5 have been combined in the graphic representations I am fully aware that this category of cases is extremely heterogeneous

Group A1

This group contains cases of chronic pyelonephritis without demonstrable obstruction of the urinary tract It also includes cases of renal papillary necrosis with concomitant symptoms of chronic pyelonephritis If a renal papilla or other anatomic impediment to the urinary flow was demonstrated, the patient was assigned to group B1

Three patients had roentgenologic changes suggestive of unilateral renal dysplasia (cases 45 55 64) Such kidneys are regarded to be predisposed to pyelonephritis (75) Since no impediment to the urinary flow was demonstrable and the patients fulfilled the criteria for a diagnosis of chronic pyelonephritis they were included in this group The series contains only one patient with diabetes mellitus (case 30) She was assigned to group A1 since she fulfilled 7 of the 8 cardinal criteria Examination of a renal biopsy specimen disclosed slight glomerular lesions and fundus changes of the type seen in diabetics were found at ophthalmoscopy Her blood pressure was slightly raised and both her blood sugar and urinary sugar were maintained within normal limits by insulin therapy

Special mention can be made of certain patients who fulfilled only three of the diagnostic cardinal criteria In cases 35 and

CHAPTER V

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calc nos s In th s group as well hypercalcae m a may have reduced the renal concentra t on ab l ty (*cf* group B1)

Gro p B3

All pat ents in th s group had arter al hypertens on All but one had stenosis of the ma n renal artery bilateral in one (case 126) The except on (case 117) had an aneurysm of both ma n renal arter es S nce operat on was be ng cons dered she was adm tted to the Renal Cl n c for b lateral nvest gat on of renal funct on includ ng Howard s test as a pos t ve result of th s test strengthens the nd cat ons for operat on (*cf* Chap VI) She was n e t gated tw ce at an interval of 8 months on the second occas on after opera t on of the aneurysm of the r ght ma n renal artery

S x of the pat ents underwent operat on for the renal artery stenos s but I d d not make any detailed study of the results of operat on regard ng such factors as the ana tom c cond t ons pressure grad ents and effect on arter al hypertens on When opera t on was performed, and ver f ed the d ag no s th s s noted in Table IV

As po nted out in Chapter VI stenos s of a renal artery — due to *e g an athero-*matous plaque — may be present w thout aus ng h pertens on (209)

Non of the pat ents n th s group ful f lled the m n mum th ee card nal criter a for a d agno s of chron c pyelonephr t s It mu t however be po nted out that these pa t en were not as thoroughly tested n th s respect as the others As a rule they were t s ed for only 5 of the 8 card nal criter a

C np B4

I ollected n th s group pat ents n whom there was rea on to presume that the renal damage was homogeneous w th l ttle or no asymmetry b tween the kidneys The d seases in quest on were renal pap llary necrosis w thout s gns of urinary tract infect on or chron c pyelonephrit s (2 cases) essent al hypertens on (4 cases) chronic glomerulone phrit s (5 cases) and the nephrot c syndrom (5 cases) Although case 145 fulf lled three of the card nal criteria of chron c pyelone phr t s a renal b opsy spec men showed sl ght glomerulonephr tic les ons It was not however poss ble to rule out uncond t onal ly the ex stence of super mposed chron c pyelonephr tis

Gro p B5

The pat ents ass gned to th s group were invest gated because chron c pyelonephrit s was suspected They all had urinary tract symptoms referred to the bladder and or urethra and could not therefore be regarded as healthy even though the r renal funct on appeared to be largely normal and they d d not fulf l the cr ter a of chron c pyelone-phr t s

In a ser es reported by another author cases in wh ch an acute exacerbat on had occurred in close assoc at on w th the tests were excluded (10b) Th s may be just fied when the degree of funct onal impa rment is to be invest gated In the present study — n wh ch the object was to compare the func t on of the two kidneys w th respect to ex st ng patholog c processes — such cases were on the contrary regarded as h ghly su table Nor d d I om t any pat ent on account of ad anced age It is true that renal funct on tests made n elderly persons often show decreas d values (151 264) However my n erest was not pr marily focused on renal funct on n general but rather on th

39, the inflammatory process was of such long duration that there were no longer any signs of activity (*e g* rise in temperature pain in the flank, bacteriuria and positive sediment) In these cases the diagnosis was verified at autopsy Cases 36 and 48, in which only three criteria were fulfilled, comp ised borderline cases between acute and chronic pyelonephritis It was difficult to decide whether it was a question of an acute, isolated attack of pyelonephritis or an exacerbation of a chronic course

Group A2

The patients assigned to this group were those with acute or recurrent acute pyelone phritis, and those suspected to have chronic pyelonephritis but who did not fulfil even the three criteria regarded as a minimum for this diagnosis Obviously, it cannot be ruled out that some of those considered to have acute pyelonephritis may have had renal damage of a chronic nature, but that any such functional defect was masked by the well maintained total function

Group B1

The patients in this group fulfilled at least three cardinal criteria of chronic pyelone phritis, but had some complicating disease of the urinary tract, *e g* urolithiasis, hydronephrosis or uretero vesical reflux

Case 103 had hyperparathyroidism with renal calculi This patient was investi gated before parathyroidectomy (as was case 109 in group B2) Hyperactivity of the pa rathyroid ho mone is known to produce an increase in both glomerular filtration rate and renal blood flow (98) In acute hyper calcaemia in animals injection of parathyroid hormone reduces the maximal renal concen tration ability (73) and hypercalcaemia is associated with hyposthenuria (160)

This group also contains six patients with renal papillary necrosis (cases 84, 86, 92, 94, 95, 105) In these cases, the sloughed papillae formed an impediment to the urin ary outflow

In case 91, *chronic pyelonephritis* was complicated by previously operated on peri nephritis on the left side

None of the cases diagnosed as chronic obstructive pyelonephritis fulfilled less than three cardinal criteria

Group B2

The patients assigned to this group had renal disease which could be expected to be unilateral, or predominantly asymmetric even if both kidneys were affected Among them were cases of congenital hypoplasia of one kidney (cases 106 112), unilateral hydrone phrosis (case 110), unilateral nephrolithiasis (case 111) and bilateral nephrolithiasis associated with hyperparathyroidism (case 109) None of the afo ementioned patients fulfilled the three cardinal criteria regarded as a minimum for a diagnosis of chronic pyelonephritis

In the remaining cases three or more diagnostic cardinal criteria were fulfilled In two patients (cases 108 and 116) coinci dent chronic pyelonephritis and silent glome rulonephritis could not be ruled out (*cf* Chap III) Two patients had concomitant renal tuberculosis and chronic pyeloneph itis (cases 113 and 114)

Finally one patient had suspected renal tubular acidosis (case 115) She had no haematologic changes characteristic of this disease nor a high urinary pH However pyelography disclosed intrarenal calcinosis denoted by the radiologist as typical of renal tubular acidosis In addition she had chronic pyelonephritis possibly secondary to the

in several of them, the investigations were made both before and during treatment In most cases long term medication was initially confined to sulphamethizole (Lucosil® Lundbeck) or to a combination of sulphamethizole and sulphamethoxypyridazine (Sulfapral® Astra) or in some cases to nitrofurantoin (Furadantin® Pharmacia) or penicillin (Cetacillin® Doktacillin® Astra) Periodically this medication was combined with antibiotic therapy after determination of the sensitivity of the invading organisms (42 44 45 104 183 184 186)

In the majority of cases treatment produced marked improvement, both objective and subjective A few patients became worse during the observation period with an associated deterioration in the results of the functional tests (*cf* Chap X pp 00—00) Consequently in some of the patients who underwent repeated tests the results varied greatly on the different occasions In these cases I considered it most correct — as can be inferred from Table X — to record them in the plots so that each test occasion was represented by one value This is why in most of the figures the number of values exceeds the number of patients

functional difference between the two kidneys It seems unlikely that the limitation of renal function which appears more or less consistently in elderly persons would affect one kidney more than the other Possible exceptions are cases in which renal function is impaired on the grounds of local anatomic changes, *e g* enlargement of the prostate Such cases have therefore been assigned to group B1

There can scarcely be any doubt that the diagnosis was, in fact, correct in the cases classified as *chronic non obstructive pyelonephritis* (group A1), even if none of the cardinal criteria alone is specific to the disease Moreover, it is improbable that these patients, all of whom were thoroughly investigated had any other intercurrent diseases than those listed in Table IV

In groups B1—B5 as well, the relevant diagnoses can be regarded as confirmed by the existence of characteristic symptoms and signs As far as group A2 is concerned it consisted chiefly of patients who had acute attacks of pyelonephritis Naturally, it cannot be ruled out that some of them actually had chronic pyelonephritis, but none fulfilled the minimum number of cardinal criteria required for a diagnosis of this disease Consequently, although they have been combined with group A1, they have been given a special denotation (◇)

Thus in accounting for and discussing the results, the cases of chronic non obstructive pyelonephritis have been considered as a homogeneous group, and their values plotted in the same figures together with those in group A2 For comparison, the values in all the other groups (B1—B5) have been combined in corresponding figures This implies that the latter plots also contain a number of cases of chronic pyelonephritis However, since all these cases were complicated by renal concretions sloughed renal papillae or other obstructions to the urinary flow, I considered it more correct to account for them together with the groups of other renal diseases In view of this procedure the groups serving as comparative material with the cases of chronic pyelonephritis do, in fact, contain precisely a number of cases of this disease This was nevertheless regarded as justified, because of the dominance of the complicating factors in these cases of *secondary chronic pyelonephritis* They are easily distinguished in the plots since they are denoted by a special sign (o)

The cases combined in the plots under the heading of other renal diseases form an extremely heterogeneous group in which such widely differing conditions as, *e g* the nephrotic syndrome and renal artery stenosis are represented Consequently it must be emphasized that when this group is discussed, it is only as a group of patients with renal diseases other than chronic pyelonephritis, or those in whom pyelonephritis was not the salient feature Obviously the combined group is not representative of any particular type of functional damage On the other hand certain of the individual groups can provide an adequate basis for discussing the implications of the results from the diagnostic and pathophysiologic aspects in the group in question This applies especially to the well defined, thoroughly investigated cases of stenosis of the main renal artery (group B3) Such a discussion is however beyond the scope of the present study For this reason only a brief account is given in the relevant connexions of the results in this group and in the other ones

With respect to the patients with chronic pyelonephritis it must be pointed out that

constriction of the main renal artery to various split function tests Howard *et al* (106) and Connor *et al* (56) stated that in stenosis of the renal artery the urinary volume should be reduced by at least 50 per cent and the Na excretion by at least 15 per cent to motivate further investigation or exploration of the kidney Both these *criteria* had to be fulfilled for Howard s test to be denoted as positive

Hradkova & Schuck (107) calculated the tubular reabsorption of water in children by *determining the concentration index of* Na $\frac{U_{Na}}{P_{Na}}$ (*cf* Chap II) No difference was noted in this respect between a group with normal renal function and a group with anomalies of the urinary tract nor could any difference be demonstrated between the right and left kidney However most of the children in a third group consisting of cases of chronic pyelonephritis showed a difference between the kidneys as *regards the reabsorption* of water These authors had no cases of arterial hypertension or stenosis of the main renal artery in their material

It is difficult to avoid leakage of *urine from the catheters and thus to make accurate* quantitative measurements of the urinary volume Rapoport (202) among others therefore suggested using the tubular *rejection* fraction in which he calculated the urinary excretion of endogenous Na and creatinine as well as the concentration of these substances in the blood but did not have to take the urinary volume into account Schuck *et al* (224) used a formula to calculate the excretion of H_2O and Na (*in* relation to the filtered quantity) which did not necessitate determining the exact volume of urine per time unit Birchall *et al* (21) also employed the relation between the urinary concentration of Na and creatinine and found *it to be better than clearance* tests for confirming the diagnosis of unilateral obstruction of a renal artery

Excretion of Hydrogen Ions and Ammonia

This has long been regarded as a true measure of tubular function (232 272) *Since evaluation of the ability of the kidneys* to acidify the urine necessitates loading with acidifying substances this method could be applied as a split function test in only a few of my cases

The method of Rehn & Günzburg (205) consisting of loading the hydrogen ion excretion with HCl and $NaHCO_3$ respectively was not used in the present investigation This method was further developed by Raabe (199) and Schneider (221) *In my* series the test was confined *to loading with* an acidifying salt (*ammonium chloride*) *For* further details see Chapter VII

Several authors have suggested *determining the NH_3 concentration in specimens* obtained by ureteric catheterization (96 218 220) The method has however a serious drawback *i e* it cannot be used in unilateral or *bilateral* infection with urease forming bacteria — *e g* certain *Proteus* strains — *since* these raise the ammonia concentration This implies that the method cannot be used as a generally reliable test

Clearance Tests

A *prerequisite for* making clearance tests *on* specimens obtained by ureteric catheterization *is* that the urine can be collected quantitatively per *time* unit from each kidney An account is given in Chapter VIII of the *complications* and sources of error associated with ureteric *catheterization*

CHAPTER VI

CHOICE OF METHODS FOR DEMONSTRATING ASYMMETRY

It was important for the investigation to choose methods which provided information about the functional state of the kidneys and, at the same time, could be used with satisfactory reliability for each kidney separately A brief survey is given in the following of the methods chosen Naturally, the investigation could have been extended to include other functional tests and urine analyses of interest for evaluating the symmetry of renal function One of the reasons for confining it to the tests described was that much more time and greater resources would otherwise have been needed Moreover I did not wish to include certain tests which might have influenced each other — An account is given in Chapter III of the methods used purely for diagnostic purposes, without a view to studying the symmetry

Renal Concentration Ability

Determination of the osmolarity of the urine is definitely the most suitable way of measuring this parameter The method was *presumably applied for the first time to* urine by von Koranyi (140) in 1897 The test is easy to perform and requires only an inappreciable quantity of urine Many investigations have been reported which prove the value of ascertaining the concentration ability by determining the specific gravity or osmolarity of the urine obtained by ureteric catheterization as a measure of the degree of damage to each kidney (*cf* Table II)

Creatinine Excretion

The creatinine excretion has long been used as a gauge of renal function Van Hoogenhuijze (105) seems to have been the first, in 1914, to determine the excretion in catheter specimens, after which the method *was soon adopted by other workers e g* Saheki (219) Ockerblad (178) 179) Lunoe (154) Yago (275) and Vuust (262) were among the first to describe determinations in catheter specimens of urine after administration of exogenous creatinine With reservation for the errors inherent in *the actual procedure of ureteric catheterization* the method is well suited for investigation of each kidney separately The sources *of error of the method and its limitations* are discussed in Chapter VIII

Diuresis and Sodium Excretion

These functions are obviously also appropriate for bilateral functional analyses here as well with reservation for the sources of error due to the catheterization procedure

Experimental polyuria has been tried (245) based on the observation that water loading results in polyuria when a kidney is healthy whereas diuresis is smaller when a *kidney is diseased The method is however* time consuming and therefore strenuous for the patient nor is it reliably undetrimental

*Since increasing interest has been focu*sed in recent years on diagnosing stenosis of renal arteries extensive work has been *devoted to correlating the existence of*

Among the methods that were not used in the pre ent series mention can be made of a few which were earlier applied as sepa ate functional tests

Urea Concentration

Determination of the urea concentration in urine was much used for many years as a test of the function of each kidney The method was introduced by Rovsing (215) in 1892 Urea was administered intravenously (86 138 275) or by mouth as in McLean s test (111 166 201 242) Gothgen (86) who worked with urea clearance tests for several years stated that the value of McLean s test as a measure of unilateral renal function could not be proved He observed — as did Husfeldt & Aikjaer (111) — several cases in which the disea sed kidney showed as good function as the healthy one or better This was ascrib d partly to the diuretic effect of urea

Dye Tests

Dye tests with e g methylene blue indigo carmine or phenolsulphonphthalein (phenol red) have long been the most extensively used tests of bilateral renal function The substances can be injected i v The method was originally introduced in 1892 by Kutner (111) as chromocystoscopy It implied that after inj cti n of e g indigo carmine the excretion of the dye was studied by inspec tion of the ureteric orifices through the cystoscope It was sometimes difficult to ju lg the in ens ty of colour (260) Better r ults w re obtain d when ureteric catheters were passed Hanzawa (90) compared the refraction specific gravity, decrease in freez ing point and urea concentration in urine and found the indigo carmine test to be the most accurate Hellström (95) and Chwalla (54) were more critical of this test They found that the results based on a large series of cases did not always permit conclusions on the degree and extent of the anatomic processes in one or both kidneys

Approximately the same results as with the indigo carmine test were obtained in determinations of the phenolsulphonph ha lein excretion (46 217, 249)

Although the excretion of a dye as a bilateral test of renal function has lost ground in the past few decades it is still used (161)

Radiorenography

This method which was devised by Winter (270) is obviously of great im portance for estimating the symmetry of renal function The literature is already voluminous (158)

The *electrourogram* which is a recording of the electric potentials from the renal parenchyma is also used to demonstrate differences between the function of the two kidneys (136)

Arterio venous haemoglobin and oxygen tes s Arterio-venous haemoglobin and oxygen differences have been compared with EPAH simultaneously in both kidneys in h althy subjects and in patients with various kinds of r nal disease (181)

The creatinine clearance test with administration of exogenous creatinine was introduced in 1926 by Rehberg (203) Molle *et al* (170) were the first, in 1929, to use the urea clearance as a test of renal function As early as 1930, Lunoe (154) calculated the creatinine clearance (after loading) and urea clearance in the urine of each kidney Many studies have subsequently been published, based on separate clearance determinations in various renal diseases The creatinine and inulin clearances have been used, as well as the PAH clearance (19 20, 23, 27 50, 51, 53, 94, 102, 108 137, 139, 142 145, 162, 173 175, 187, 189 214, 222, 228 261)

In recent years the improved possibilities of diagnosing stenosis of renal arteries have in fact increased the importance of separate clearance determinations (5 6 87 134 156 157 192, 226 233 238, 240 259)

Despite the sources of error inherent in separate clearance determinations they were performed in a number of cases in my series chiefly in connexion with renal vein catheterization

Renal Extraction of PAH and Diodrast

As far as earlier studies of these determinations are concerned, reference is made to Chapter VII (Renal vein catheterization)

Roentgenologic Examination

Naturally this method of examination — especially *excretory pyelography* — has been used to evaluate the state of each kidney ever since it became possible with the help of roentgen rays to visualize the contours of the kidney with the rest of the urinary tract and the renal parenchyma There is a wealth of literature on the subject, most of which is beyond the scope of the present study Pertinent references are given in Olsson s paper (182)

Mention can, however, be made of one method used for diagnosing stenosis of a renal artery Birchall *et al* (21) suggested hydrating or dehydrating the patient in connexion with pyelography The method was further elaborated by Amplatz (1) and Brannan *et al* (26) In patients with renal artery stenosis the nephrogram effect in the stenosed kidney appears later and persists for a longer time when the patient is dehydrated Pyelography has been regarded by most authors as inferior to quantitative physiologic methods for judging renal function (52 66, 67, 274)

Aortography and *renal angiography* are also methods of importance in studies of symmetry They disclose the anatomic details in the form of narrowings of the lumen their extent and degree as well as the existence of any post stenotic dilatation Several authors have however pointed out that renal artery stenosis (209) of varying degree and multiple renal arteries (61) are not infrequently demonstrated without the patient having controllable renal hypertension or a rise in blood pressure of any kind

In my cases renal angiography was sometimes of value in the diagnosis of chronic pyelonephritis as well This applied particularly to such questions as the extent of pyelonephritic scarring size of the kidney and as a differential diagnostic aid *Micturition cystography* e g when vesico renal reflux was suspected and *retrograde pyelography* were also found to be valuable complements to other roentgenologic examinations

Among the methods that were not used in the present series mention can be made of a few which were earlier applied as separate functional tests

Urea Concentration

Determination of the urea concentration in urine was much used for many years as a test of the function of each kidney The method was introduced by Rovsing (215) in 1892 Urea was administered intravenously (86 138 275) or by mouth as in McLean s test (111 166 201 242) Göthgen (86), who worked with urea clearance tests for several years stated that the value of McLean s test as a measure of unilateral renal function could not be proved He observed — as did Husfeldt & Alkjaer (111) — several cases in which the diseased kidney showed as good function as the healthy one or better This was ascribed partly to the diuretic effect of urea

Dye Tests

Dye tests with, *e g* methylene blue indigo carmin or phenolsulphonphthalein (phenol red) have long been the most extensively used tests of bilateral renal function The substances can be injected i v The method was originally introduced in 1892 by Kutner (144) as chromocystoscopy It implied that after injection of *e g* indigo carmine the excretion of the dye was studied by inspection of the ureteric orifices through the cystoscope It was sometimes difficult to judge the intensity of colour (260) Better results were obtained when ureteric catheters were passed Hanzawa (90) compared the refraction specific gravity decrease in freezing point and urea concentration in urine and found the indigo carmine test to be the most accurate Hellström (95) and Chwalla (54) were more critical of this test They found that the results based on a large series of cases did not always permit conclusions on the degree and extent of the anatomic processes in one or both kidneys

Approximately the same results as with the indigo carmine test were obtained in determinations of the phenolsulphonphthalein excretion (46 217 249)

Although the excretion of a dye as a bilateral test of renal function has lost ground in the past few decades it is still used (161)

Radiorenography

This method which was devised by Winter (270) is obviously of great importance for estimating the symmetry of renal function The literature is already voluminous (158)

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Table VII *Normal values for some tests of renal function*
The relevant values are according to reports from the Clinical Central Laboratory St Eriks Sjukhus and experience at the Renal Clinic

Serum creatinine 0 9 1 4 mg/100 ml
Renal concentration ability > 800 mOsmol/l t
Renal acidifying ability <pH 5 3
Extraction of radioactive Diodrast (whole blood) 49 86 / (16)
Extraction of PAH See Table III
Renal plasma flow (determined with PAH) 650±31 9 s 150 ml min/1 73 m² BSA (14)
PAH *clearance* 589±29 3 s ±138 ml min/1 73 m² BSA (14)
Endogenous creatinine clearance 32 healthy volunteers (18 women and 14 men aged 16 67 years)
Women (mean) 89 9±6 71 s ±28 49 ml/min 1 73 m² BSA.
Men (mean) 96 0±8 06 s 30 16 ml min 1 73 m² BSA
Inulin clearance 119 4 6 s ±14 4 ml min 1 73 m² BSA (14)

the concentrations used in arterial blood PAH in urine was determined by the same method but without precipitation of protein

No PAH determinations were made in patients who during the previous 48 hours had taken any drugs that might have disturbed the investigation (*e g* sulphonamides PAS or Palerol comp®) It has been shown that an acetylation of PAH in the kidneys if it occurs is of negligible influence in the determination of PAH (176) My series contains only one patient with diabetes mellitus and she had no glycosuria at the time of investigation This is worth mentioning since glycosuria may cause errors in the PAH determination (72)

Radioactive Diodrast was determined by gamma counting of haemolyzed blood at the photopeak of ¹³¹I 0 38 meV as described by Bergstrom *et al* (16)

Methods of Clinical Investigation

Renal vein catheterization

Cournand & Ranges (60) elaborated the method of catheterizing the heart introduced by Forssmann (79) to include other parts of the circulatory apparatus It was not until Warren *et al* (265) devised a method for obtaining blood from the renal veins in man with the help of a Cournand catheter that it became possible to determine the renal extraction of such substances that are excreted in large quantities in the urine *e g* PAH and Diodrast When the PAH extraction could be determined at renal vein catheterization this value and the renal PAH clearance could be used to calculate the renal plasma flow with much greater accuracy than before The method described by Warren *et al* (265) implies a great advance in our possibilities of studying the physiology and pathophysiology of the kidneys in man The method is however associated with certain difficulties and risks

With the technique now used which was introduced by Seldinger (227) the catheter is advanced through a femoral vein This has considerable advantages In view of several other technical improvements the method is now so simple and has such slight risks that it has become one of the routine methods at our clinic

Except in healthy experimental subjects renal vein catheterization was preceded by excretory pyelography to evaluate such factors as the size of the kidney and position of the renal hilus In addition all patients were investigated for the existence of any

4

CHAPTER VII

METHODS

The chemical analyses were performed at the Central Laboratory of the hospital, whose staff has great experience of making such determinations Some normal values for renal function are given in Table VIII

Analytic Methods

Osmolarity of urine The depression of the freezing point was determined with a thermistor and Wheatstone bridge, using a modification of the apparatus described by Bowman *et al* (23)

Creatinine Creatinine in *serum* was determined in the Folin Wu filtrate according to Haugen s modification (93) of Brod & Sirota s method (32) Creatinine *in urine* was determined directly with picric acid The method is not specific, since it does not take into account the influence of chromogens

Sodium and potassium in serum and urine were determined in an Eppendorf flame photometer (17)

Chlorides in serum and urine were determined by Brun s modification (35) of the method of Shales & Shales

Serum bicarbonate (alkali reserve) was determined by the titrimetric method of Van Slyke *et al* (231) using a Beckman automatic potentiometric titrator

Protein in urine This was determined by the biuret method after precipitation with trichloracetic acid and redissolving the precipitate in alkaline biuret reagent

pH of urine The determination was made electrometrically (Radiometer s pH meter) This was done as soon as possible after the urine was voided to obviate any change due to formation of ammonia and loss of CO_2

Ammonia and titratable acid These determinations were made in a large number of cases on bladder and ureteric urine, but since it was subsequently found that this had not been done consistently the results have not been included Moreover, the urine was sometimes strongly infected by urease forming bacteria, and determinations of the ammonia content were then regarded to be of limited interest

Inulin was initially determined by Josephson & Godin s modification (120) of Corcoran & Page s method (58) using a diphenylamine reagent Later the method of Heyrovsky (97) was used instead

PAH The technique for determining PAH was changed in the course of the investigation Initially, the method of Smith *et al* (235) was used, but was later replaced by that of Brun (36a) The two methods give compatible values When the protein was precipitated for determining the PAH in arterial blood the plasma dilution was 1 15 When determining the low concentrations of PAH in renal vein blood, the dilution was 1 10 In precipitation of protein a lower dilution cannot be used since the PAH may then be lost with the precipitate At low concentrations in renal vein blood the values were read against a blank using the method described by Smith *et al* (235) The blank is negligible in determination of

the brachial vein The infusion solution consisted of 450 ml of physiologic saline with the addition of 20—40 ml of 20 per cent PAH (In a few patients with signs of greatly impaired renal function only 5—10 ml of PAH were given) Fifty ml of a 10 per cent inulin solution were added to the infusion solution of which 20—30 ml were given as a priming dose followed immediately by continuous infusion at a rate of 2 ml minute A pump designed by Ek (71) was used for this purpose These measures allowed the concentration of PAH in plasma to be kept on a fairly constant level of 1—4 mg/100 ml, and that of inulin at about 50 mg/100 ml

To check that the catheter in the renal vein was in the proper position during catheterization a rapid approximate method was used to determine the PAH concentration in heparinized whole blood from the renal vein and arterial blood When the preliminary analyses showed no definite difference in concentration it was considered probable that the catheter tip did not lie in the renal vein its position was then checked and adjusted if necessary As a rule 5 to 10 determinations were made on blood from one or both kidneys at intervals of 5—15 minutes

In some cases a solution containing 0 15 ml/kg body weight of a 20 per cent solution of the sodium salt of PAH *mixed in the* syringe with 0 05 ml/kg body weight of 2 per cent Xylocaine epinephrine® was administered 1/2 to 1 hour before the examination The solution was given as a slow intramuscular injection in the superior outer quadrant of the buttock as described by Bucht (40) A smaller quantity of PAH was given to patients with impaired renal function

To permit samples to be taken of the peripheral blood a polythene catheter was inserted in the brachial artery The i v infusion was given through a plastic tube introduced into a cubital vein without local anaesthesia whereas arterial puncture was preceded by local anaesthesia with 2 per cent Xylocaine®

Titration of the depression limit for renal extraction of PAH In all essentials the method used was that described by Bergstrom *et al* (16) Briefly it implies that a catheter was passed into a renal vein and that repeated determinations of the renal extraction of PAH were made while the PAH concentration in arterial blood was increased stepwise As a rule it was tried to start at 1—3 mg/100 ml serum, after which the determinations were repeated at about 5 10—15 and 15—20 mg/100 ml Little regard was paid to PAH clearance since clearance determinations are of slight or no value with a varying high PAH concentration in plasma In some cases bilateral determinations were made simultaneously

Josephson *et al* (121—123) found that Diodrast and PAH competed with each other with respect to tubular excretion as has been shown to apply to other pairs of substances (232) They presumed that Diodrast and PAH are excreted by means of a common enzyme system or at any rate by an enzyme system in which some step must be in common Consequently it could be expected that the extraction of Diodrast would also start to rise when the PAH extraction was increased so that its depression limit was reached In the experiments 0 10—0 25 μC/min (totally 25—35 μC) of carrier free radioactive Diodrast (Abbot) was given in the same infusion solution as inulin and PAH Since the extraction was calculated from the

cardiovascular disease, by means of physical and roentgenologic examination of the heart, diurnal recording of the blood pressure, and ECG recording Since renal vein catheterization places little strain on the patient and is done under local anaesthesia poor general condition was considered to be the only contraindication

Initially renal vein catheterization and ureteric catheterization were combined (39 investigations), but this procedure was subsequently abandoned, as being too troublesome for the patient The combined examination was, however, made in patients with arterial hypertension, when this was suspected to be of renal origin In these cases, fluids were not restricted In the other cases, the bladder urine was collected through a catheter in the bladder at renal vein catheterization (50 investigations) and ureteric catheterization was performed as a separate examination (116 investigations) The technique of renal vein catheterization has been described in detail by Edvall (70), but a brief account is given in the following since certain modifications were made in the present study

As in Edvall s technique, the catheter was introduced through the femoral vein The site of puncture in the inguinal region (usually on the right side) was infiltrated with 20—30 ml of 0 5 per cent Xylocaine® after palpating the femoral artery The needle used for puncture of the femoral vein was the curved cannula with mandrin designed by Gidlund (83) When the needle had been advanced for a short distance towards and through the wall of the vein the mandrin was removed and the patient instructed to bear down When venous blood was expelled under pressure a metal guide of Seldinger s model (227) was introduced All manipulations of the guide and catheter were made with great caution, since incautious contact with the vessel walls may elicit a vasovagal reaction with a resulting fall in blood pressure

When the metal guide had been advanced for a few decimetres the puncture needle was withdrawn and a catheter introduced over the guide This catheter was of radio paque material (180) and curved before hand (83), with a diameter of curvature of 3—4 cm It was advanced, under fluoroscopic control beyond the site of origin of the renal vein after which the guide was withdrawn The catheter was then passed slowly downwards by moving the tip, and usually slid easily into the renal vein In some cases a catheter was inserted into each of the renal veins to obtain simultaneous samples Both catheters were introduced through the same puncture hole

The movements and position of the catheter (or catheters) were checked continuously on a television screen, which reproduced the roentgenologic picture of the current position of the catheter Initially a Philips Image Amplifier was used for fluoroscopy during catheterization but this required the examination to be made in almost complete darkness and in an uncomfortable position for the examiner Consequently it was a great advantage when the picture could be reproduced on a closed circuit TV screen (Philips TTV chain) placed near the patient s head This implied that the image was clearer and the examination could be carried out in subdued room illumination Moreover this method requires a much lower intensity of roentgen irradiation

In most cases PAH was administered as an i v infusion through a plastic catheter in

Initially ureteric catheterization was combined with renal vein catheterization (*cf* p 50) In the majority of cases however it was done at a separate session

Premedication consisted of 20 mg of 1 N butylscopolammonium bromide (Buscopan ®) or 80 mg of papaverine hydrochloride injected i m Sometimes a short acting barbiturate (5 ethyl 5 isoamylbarbituric acid Pentymal®) in a dose of 0 1 g was given as well No drugs were administered while the examination was being made

After local anaesthesia of the urethra with Xylocaine gel® a Brown Buerger cystoscope (24 Fr) was introduced with great caution The ureteric catheters were of 5—7 Fr calibre and had lateral holes about 1 cm apart arranged spirally in the first 10 cm from the tip (154) If possible the catheters on both sides were of the same calibre They were advanced 12—15 cm in the ureters The cystoscope was then withdrawn, and a bag catheter (Foley 16—18 Fr) was passed into the bladder to check that no urine leaked along the sides of the ureteric catheters In some cases a check was made of possible leakage by means of chromocystoscopy and inspection of the ureteric orifices as recommended by *e g* Rothauge (214) and Vondra (261) I injected a dye i v and then observed through the cystoscope left in the bladder whether any coloured urine escaped from the ureteric catheters I did not use suction as suggested by Lunöe (154) in view of the risk of the catheter adhering to the wall of the ureter (102)

Collection of urine When the catheters were in position the bladder was thoroughly emptied by means of irrigation with physiologic saline followed by insufflation of air This was done to enable any leakage to be checked as well as to prevent the renal haemodynamics and function from being influenced by distension of the bladder by urine (193) The first few millilitres of urine from the ureters were discarded since it could not be ruled out that they contained bladder irrigation fluid Actually I waited for 15—20 minutes before starting to collect specimens for analysis since the first portions of urine are regarded to be more affected by the manipulations than are later portions (*cf* Chap VIII) The mouths of the ureteric catheters were placed as low as possible to take advantage of the siphon effect

The urine was collected simultaneously from each kidney in graded tubes which were changed concurrently and the time carefully noted Quantities ranging from 1 to 8 ml were required for the analyses Several specimens were collected from each ureter and the mean value of determinations of *e g* osmolarity electrolyte content and clearance was taken as the basis for evaluating the function

The urinary bladder was carefully emptied after each period The first specimens from each ureter were taken under sterile conditions for bacteriologic examination as was urine from the bladder in connexion with cystoscopy Before the catheters were withdrawn the patient was usually given a spasmolytic and or analgesic agent

Complications The results were discarded, and the examination was sometimes repeated, if any of the following events occurred

— The urinary flow was so small that a sufficient quantity could not be collected for the analyses

— The urine contained an appreciable amount of blood

— There was poor agreement between the results in the different periods

number of counts in blood from an artery and the renal vein, it expresses the extraction in whole blood and not in plasma, as in the case of PAH

In the present investigation I used the method of Josephson *et al* (121, 122) as modified by Bergstrom *et al* (16) On technical grounds, the renal extraction of radioactive Diodrast was determined in only five cases, in one of them bilaterally (Fig 16) This case is given as an illustration of an attempt to check the result in studies with varying PAH extraction

Complications In my series, complications which necessitated discontinuing the examination occurred in only two cases Thus, in the 131 renal vein catheterizations in which bilateral determinations were made (89 catheterizations), a haematoma in the groin with a transient fall in blood pressure occurred in one patient, and another also had a temporary fall in blood pressure, probably caused by vasovagal reflex (case 2) The former patient was excluded from the material, whereas the results of tests in the latter patient were included up to the time when the first reaction was observed

To decrease the risk of haematoma, slight pressure was applied to the site of puncture during the examination When the catheter had been withdrawn, pressure was applied for a few hours by means of sandbags No signs of thrombosis of the punctured vessel nor of embolism were observed (152)

Ureteric catheterization

Interest in studying the function of one kidney in relation to that of the other has existed for a long time The work of Nitze & Casper (145), performed around 1890 led to ureteric catheterization being used more often than before as a method of examination As early as about 1902, Nitze constructed a ureter occluding catheter with a rubber balloon which surrounded the catheter and was filled with water after the catheter had been passed (145) The construction was, however, so clumsy that it was difficult to use Attempts have also been made to divide the bladder into two halves by a so called vesical separator, but this technique is best suited to animal experiments (27, 145)

Others have succeeded in occluding one ureter by external pressure (19), and collecting urine from the other ureter by passing a catheter into the bladder Some authors (65, 131, 149 214) have passed only one ureteric catheter the urine from the other ureter being collected in the bladde , and led off through a cystoscope or vesical catheter (51, 55, 145, 149 187, 202, 233)

A somewhat varying technique has been used for ureteric catheterization As a rule the catheters have been of relatively large calibre (5—9 Fr), and often provided with lateral holes in the tip They have been advanced into the ureter for 15—25 cm Ureteric catheters with lateral holes were introduced by Lunoe (154) He made a spiral pattern of 10—12 lateral holes about 1 cm apart in the first 10 cm of the catheter

In the present series, the procedure was as follows All chemotherapeutic or antibiotic therapy was discontinued when the patient was hospitalized for investigation Renal vein and ureteric catheterization as well as the other examinations were performed as soon as possible Treatment of any urinary tract infection on the basis of the antibiotogram, was postponed until immediately after catheterization

Initially ureteric catheterization was combined with renal vein catheterization (*cf* p 50) In the majority of cases however it was done at a separate session

Premedication consisted of 20 mg of 1 N butylscopolammonium bromide (Buscopan ®) or 80 mg of papaverine hydrochloride injected i m Sometimes a short acting barbiturate (5 ethyl 5 isoamylbarbituric acid Pentymal®) in a dose of 0 1 g was given as well No drugs were administered while the examination was being made

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— The urine contained an appreciable amount of blood

— There was poor agreement between the results in the different periods

— The catheter became occluded, and patency could not be restored by any of the following measures

To restore passage in the catheter when the flow was arrested, 1—2 ml of air were insufflated Neither distilled water nor physiologic saline was used, since this would have disturbed the determinations Sometimes a blood clot was expelled, after which the flow started again In some cases the catheter was exchanged for another one If patency was not restored despite these measures the ureteric catheter was withdrawn and the urine from the relevant side was collected in the bladder, and allowed to flow out through the vesical catheter In a few cases the examination was discontinued

Since the complications associated with ureteric catheterization are well known, they need not be discussed further It must, however be mentioned that such complications arose on a few occasions in the present material For example bilateral occlusion of the ureteric catheters occurred in one case, due to the presence of blood clots, with resulting oliguria which was subsequently relieved This case is not included in the results

Many patients experienced transient pain during catheterization and sometimes after it as well These cases have not been excluded Nor were any cases excluded on the grounds of mild bleeding Nevertheless, it cannot be ruled out that the inappreciable complications observed in some patients in the form of pain and sometimes a slight fall in blood p essure may have influenced the results (*cf* Chap VIII)

I found no signs of cystoscopy or ureteric catheterization having elicited an ascending urinary tract infection in any case This is in agreement with the experience of several authors (8 113 216 253), although others maintain that there is a certain risk of this complication (155 171, 204, 230)

No prophylactic antibacterial treatment was given, but therapy was generally instituted immediately after this examination, on the basis of antibiotograms made on specimens of urine taken shortly before it

Endogenous creatinine clearance

This was determined as the apparent creatinine clearance, and was generally done in conjunction with determination of the PAH clearance This applied both to the 2 periodic determination and to that made in connexion with renal vein catheterization In a few cases the 24 hour clearance of creatinine was determined

Inulin clearance

This was determined only in the patients who underwent renal vein catheterization The inulin was administered in the same infusion solution as PAH The quantity given was calculated to achieve a plasma concentration of about 50 mg/100 ml Initially, a Czech inulin was used, later replaced by a German one and recently, by an American preparation

PAH clearance renal plasma flow and PAH extraction

PAH clearance As already mentioned this determination was often combined with that of endogenous creatinine clearance At 6 a m the patient drank 2 glasses of water and 1 hour later the same quantity Fasting was not required but smoking and drinking of tea or coffee were not allowed If the patient was being treated with sulphonamides PAS or tropenzilium bromide piperylon maleate (Palerol®) this medication was discontinued for a few days before the test

The test was started at about 8 a m when the patient was given PAH in a dose related to the body weight as an intramuscular injection together with Xylocaine norepinephrine® (10) The clearance determination was started about 1 hour later As a rule two consecutive periods of about 60 minutes each were determined After each period the bladder was emptied as completely as possible and a venous sample was taken The patient was not catheterized unless she had difficulty in emptying her bladder or was menstruating In a few patients — chiefly men — the duplicate determinations showed a lack of agreement For this reason the values for PAH and endogenous creatinine clearance are listed only when the difference between the periods was less than 25 per cent of the higher value

The PAH clearance was sometimes determined in connexion with renal vein catheterization with both ureteric catheters in place The patient was then deprived of fluids for 24 hours before the test since ureteric catheterization was combined with the test of renal concentration ability In these cases PAH was given together with inulin as an i v infusion at a constant rate as previously described In these cases the renal vein blood was analyzed in a way that allowed the extraction of PAH to be determined It was therefore regarded as unimportant if this concentration exceeded the depression limit in patients with impaired renal function Obviously if this occurred the clearance value was worthless

Renal plasma flow This was determined in the same way as the PAH clearance but by analysis of renal vein plasma

Renal PAH extraction This was calculated from the concentration of PAH in arterial and venous blood

Renal extraction of radioactive Diodrast

This was determined by the method of Josephson *et al* (121, 122) as modified by Bergström *et al* (16) cf pp 51—52

Renal concentration ability

The test was made after the patient had been given a dry diet and deprived of fluids for 24 hours Vasopressin (Pitressin tannate® in oil) in a dose of 5 I U was injected i m at 8 p m in the evening before the test The osmolarity was determined in the urine voided at 8 and 10 a m on the following morning The patient was prepared in the same way when the test was made with catheters passed into the ureters to determine the urinary concentration ability of each kidney separately

Water loading test of Volhard

No fluids were allowed after 8 p m in the evening preceding the test At 8 a m on the following morning the patient urinated was weighed and then drank 1 litre of water as quickly as possible without discomfort The patient then emptied his bladder at intervals of 1 hour for the next 4 hours after which he was weighed again The quantity of urine was measured and the osmolarity determined as soon as was feasible In no case did the water load cause nausea or other signs of water intoxication

Renal acidifying ability

This test was made only in cases in which there was considered to be no risk of metabolic acidosis The patient was given ammonium chloride in a dose of 0 1 g kg of body weight per day on 3 consecutive days The daily quantity was divided into three doses given in the form of tablets The pH and content of ammonia and titratable acid were

determined in the first urine specimen on each day The last specimen tested was the 4th day's morning urine The specimens were preserved with one drop of toluene When pH, ammonia excretion and titratable acid were determined in catheter specimens from the ureters, the test was sometimes preceded by acidification as just described When this was done, it is noted in the tables

Renal biopsy

This was performed with a modified Vim Silvermann needle using the technique of Kark & Muehrcke (126) The specimen was transferred to formalin, and sent to the Department of Pathology for examination

Urinary sediment

In some cases, the technique suggested by Sternheimer & Malbin (243) was used to stain the leukocytes, with their characteristic, large vacuolated cells, which are pale, bluish in colour and of varying shape In addition, a semi quantitative determination was made of the cells in a given volume of sediment from the 24 hour urinary output The microscopic evaluation of the sediment in all cases of pyelonephritis was made by the same experienced examiner, before the patient had been given any therapy or had undergone any investigations

Urine culture

This was done in all patients and a quantitative bacterial count was made in most of them In men a mid stream specimen was collected, after washing directly in a sterile container Since the end of 1959 women patients have, with few exceptions emptied their bladder through a sterile glass tube 120 mm long and 12 mm in diameter (45) held against the urethral orifice by the patient or an attendant A mid stream specimen was then taken A specimen collected with a tube held against the urethral orifice like a vesical catheter specimen, raises the bacterial count only inappreciably, whereas in women an ordinary mid stream specimen often gives bacterial growth, partly due to contamination from the external genitalia or urethra In the few cases in which the glass tube method was impracticable, a mid stream specimen was taken after thorough washing, being careful to avoid the urinary stream coming into contact with the labia majora

In quantitative counts of the bacteria serial tenfold dilution plate counts were used whereas in non quantitative culture the urine was undiluted The borderline for significant bacteriuria (> 100,000 bacteria/ml urine) applies only if the patient is not being treated with chemotherapeutics or antibiotics A number of additional factors must be taken into account when evaluating the results of culture, e g pH and dilution of the urine, ureteric obstruction — which may give a false negative result — and time of taking the specimen (first morning specimen or random specimen) To decrease the sources of error in the present investigation, the procedure for taking the specimens was standardized, and they were transported directly in the morning to the laboratory where they were dealt with at once

In almost all the patients, culture of morning urine on Lowenstein medium and inoculation of guinea pigs for detection of tubercle bacilli were performed on 3 consecutive days

Blood pressure determinations and examination of ocular fundi

A 24 hour curve for the arterial blood pressure was generally recorded on the 1st

or 2nd day of hospitalization The measurements were made at approximately 2 hour intervals with the patient confined to bed Unless otherwise noted in the tables all hypotensive therapy had been discontinued for at least 5 days before the recording

The ocular fundi were inspected the stages of retinopathy (I—IV) being defined according to Keith & Wagener

Roentgenologic Examination

All these examinations were carried out in the Department of Roentgenology of the hospital under the supervision of experienced roentgenologists

In *excretory pyelography* the contrast media used were three iodo compounds (Hypaque® Winthrop or Urografin® Schering) *Retrograde pyelography* and or *urethrocystography* were performed in a few cases and one of the aforementioned contrast media was generally used The ureteric catheter for retrograde pyelography was of 5—7 Fr calibre and had only one or two holes in the tip It was advanced into the renal pelvis In some cases serial radiograms with a film changer (83) were made during slow withdrawal of the catheter and concurrent injection of contrast medium In this way it was sometimes possible to visualize the changes in the renal pelvis or ureter responsible for poor opacification of the kidney at excretory pyelography

Selective renal angiography was performed by the method of Seldinger (227) using an Odman catheter (180) bent according to Gidlund (83)

Calculation of Results and Statistical Methods

Conventional statistical methods were used

When evidently erroneous results of extraction determinations had been excluded (cf p 63) the mean was recalculated from the remaining values As far as the renal extraction of PAH is concerned this applied to 5 determinations In these cases certain of the erroneous values are probably to be ascribed to the renal vein blood being mixed with blood from the vena cava It cannot however be completely ruled out — even if such an explanation seems highly unlikely — that a lower value for PAH extraction might have been due to the renal vein blood coming from a part of the kidney where the PAH extraction was lower in view of more extensive parenchymal damage If this was actually the case it implies that the procedure of exclusion introduced an error in evaluation of the results

When calculating the mean of clearance and RPF determinations with more than one period the values for each period were weighted against each other taking the time factor into account When the total clearance was calculated for both kidneys these values were correlated to a body surface area of 1 73 m^2 This conversion was not however done when a comparison was made between the clearance by the right and the left kidney since the percentage difference between them was calculated

When a *single* determination was made of the osmolarity sodium, potassium and creatinine concentration in the urine from one kidney only the value for the corresponding period on the other side was included

Recording of symmetry and asymmetry

When estimating the way in which renal disease had affected the symmetry of renal function I started out by comparing the size of the relevant parameters on either side

Such a comparison is however, apt to be misleading This is because a difference which appears inappreciable when the kidneys are functioning normally may, in fact, represent a great discrepancy in function in severely damaged kidneys, where *e g* extraction of PAH, and excretion of water, electrolytes, PAH and creatinine may be greatly decreased Consequently, when accounting for the results, I have given most of the differences — not in absolute figures — but as the percentage difference between the kidneys as a measure of the asymmetry The difference was calculated as a percentage of the function of the better kidney, measured by the same parameter, and not as a percentage of the mean value, as described in Chapter I

It would perhaps have been more correct to base the calculation on the functional impairment in the poorer kidney, and thus to have obtained a more accurate measure of how far the disease had advanced However, when the difference is calculated as a percentage of the function of the better kidney, the range will naturally be smaller than if the corresponding calculation had been made on the poorer kidney, since the maximum difference cannot exceed 100 per cent

SOURCES OF ERROR OF THE CLINICAL PHYSIOLOGIC METHODS

General Physiologic and Analytic Aspects

When a comparison is to be made between the two kidneys particular regard must naturally be paid to the reliability of the methods used Actually the battery of renal function tests is relatively limited and only certain details of renal function are amenable to testing Furthermore none of the tests that can be applied in an investigation of this type is selective for a given function even if some of the tests are influenced chiefly by an isolated partial function

The fact that so many methods have been used to investigate patients with renal disease indicates that none of them is completely satisfactory Often the healthy kidney compensates fo the insufficiency of the diseased one to such a great extent that, if only the total function is measured it is found to be normal and the state of the diseased kidney is masked in this way (163)

To study the functional capacity of the proximal tubules I used in the first place the *renal extraction of PAH* Since the two kidneys are to be compared it is of minor importance whether or not the plasma concentration of PAH is kept on a low and constant level (4 mg 100 ml)

The function of the distal tubules and collecting tubules was studied by *means of* the *renal concentration ability* expressed as the highest osmolarity of the urine determined as described in Chapter VII However in view of the errors that are apt to arise in quantitative collection of urine during catheterization of a ureter I have not given any quantitative data for the total excretion of individual electrolytes Instead I have confined myself to giving the concentration in the specimens obtained from both ureters concurrently The results of clearance tests are exceptions The sources of error have in fact proved to be negligible in determinations of the osmolarity of the urine by cryoscopy as well as in analyses of the urinary concentration of sodium and potassium by flame photometry (13 117)

Obviously tubular function can be studied in other ways On practical grounds I limited myself to the methods described except in a few cases in which determinations were made of the excretion of titratable acid and ammonia by each kidney separately However since only a few cases were involved and the determinations were not made consistently no conclusions are permissible

As already mentioned in Chapter III an additional reason for not including these determinations was that some patients have a tendency to metabolic acidosis which may easily develop from latent *into manifest* acidosis on loading with an acidifying salt Urine heavily infected by urea splitting bacteria — *e g* some *Proteus* strains — is another complicating factor ammonia is then released and gives false values for the concentration of both ammonia and titratable acid When the pH of the urine was determined in connexion with passage of ureteric catheters it was impracticable for the test to be preceded by acidification For this reason, reservations must be made for the

value of this determination The pH values are nevertheless included in the tables and the results are compared with those of other selective renal function tests

At our clinic, the *endogenous creatinine clearance* is used routinely for a study of the glomerular filtration rate The creatinine is determined without any absorption procedure, *i e*, without taking into account the chromogens which, like creatinine, give a colour reaction with picric acid It has been shown by several authors that the chromogens present in plasma result in the creatinine value being somewhat higher than that representing the true creatinine (118) It has also been demonstrated that when the creatinine concentration is high, some part is excreted through the tubules, and it seems probable that it can also be reabsorbed to some extent in the tubules (12) Consequently, the endogenous creatinine clearance, as determined in this investigation, is only an approximate measure of the glomerular filtration rate, and is associated with considerable sources of error It appears likely that the discrepancies caused by these errors are symmetric in healthy kidneys, as shown *e g* by Haugen s results (94) Naturally the error introduced by the chromogens in the plasma affects the clearance value for each kidney to the same extent

This argument applies not only to determination of the endogenous creatinine clearance but also to analysis of the *creatinine concentration* in the urine from each kidney, which was also used to study the existence of any asymmetry of function

In many of my cases the *inulin clearance* was also determined using current methods This clearance value is in all probability, as close as we can come at present to the true glomerular filtration rate Nevertheless it is not absolutely unquestionable that the inulin clearance too does not differ, to some extent, from the true filtration value As early as 1945, Lindahl & Josephson (150) demonstrated that certain kinds of inulin were not recovered quantitatively in the urine of man after i v injection This might indicate that certain inulin preparations are metabolized to some extent in the organism Balint (7) for example stated that he had found indications that inulin can be accumulated in the kidneys of dogs Experiments now in progress at our hospital also suggest that inulin clearance is not always identical with the glomerular filtration rate

In my series, the *renal plasma flow* was studied chiefly by means of PAH Determination of the PAH clearance is obviously associated with the same sources of error as other determinations of the clearance by each kidney separately The PAH clearance is known to be of no value for estimation of the renal plasma flow or renal blood flow in damaged kidneys, in which the renal extraction is reduced In my cases however, the PAH concentration was always determined in blood from the renal vein as well which largely eliminated this source of error Other sources of error nevertheless remain such as possible accumulation of the test substance in the renal parenchyma (7 119 122 123) and passage of PAH into the erythrocytes (121) It can however be presumed that these sources of error are of little consequence

It is obvious that *roentgenologic examination* of the kidneys — apart from being one of the foremost diagnostic aids — is also a handy valuable method for studying the symmetry and asymmetry of renal function In hardly any case in the present series

did there appear to be a discrepancy between the results of roentgenologic examination and the functional tests Since the roentgenologic examinations served merely as a standard of reference for the symmetry of renal function their value will be only briefly mentioned here

As a rule excretory pyelography gives a clear picture of the anatomy of the kidneys provided that renal function is such that a sufficient density of contrast medium can be obtained (*cf* Chap I) On the other hand excretory pyelograms of the kidneys are inferior to quantitative determinations of renal function for assessing the degree of functional impairment (52 66 67 274 *cf* Chap VI) This is particularly true of renal artery stenosis in which Morris & DeBakey (172) reported that 30 per cent of their patients presented normal pyelograms The results in this category of patients in the present study largely agree with this observation

The interval between pyelography and ureteric catheterization should be as short as possible one of the reasons being to establish whether there is any obstruction of the urinary tract

Although determination of the *bacterial type and bacterial count* in the urine from each kidney is also of some diagnostic value these determinations have several technical limitations *e g* the interval before cultivation can begin (the number of bacterial colonies doubles in about 20 minutes) and the effect of anatomic obstructions on the urinary flow A difference between the incidence of bacteria in the urine from the two kidneys cannot be said to have been established unless — in untreated cases — the bacterial count per unit volume of urine is at least 10 times greater on one side The borderline for significant bacteriuria has been drawn at 10 000 bacteria/ml urine For bladder urine the corresponding value is 100 000 bacteria/ml (127 *cf* Chaps I and III) These limits serve as satisfactory screening tests but must be lowered if the patient is undergoing treatment

Effect of Renal Vein Catheterization on the Determinations

When a comparison is to be made between the extraction by the two kidneys variations in the anatomic and topographic conditions may play a certain role

If the catheter tip lies far out in the renal vein there is a risk that blood may be collected only from a limited part of the kidney — *e g* from a greatly damaged part of it from a relatively undamaged part or from a healthy part If the renal damage is homogeneous this may not have any great effect on the symmetry of the values but if renal function is inhomogeneous the magnitude of this source of error cannot be estimated In the present investigation I tried to avoid advancing the catheter tip so far that this source of error would come into effect

On the other hand, Edvall (70) demonstrated that the catheter tip cannot lie juxtacavally or centrally in the renal vein without the risk of admixture of blood from the vena cava and the tributaries (testicular/ovarian and suprarenal veins) although the latter applies only to the left renal vein Smith (232) stated in fact, that renal vein catheterization should not be performed on the left side in view of this likelihood of admixture Edvall (70) showed that the catheter tip should be advanced at least 2 cm in the left renal vein to eliminate the

risk of mixing with blood from tributaries, as well as backflow from the vena cava However, Johnstone (116), who made autopsy studies in 10 subjects with healthy kidneys, found that the distance between the opening of the suprarenal vein into the left renal vein and the vena cava was a mean 3 19 cm (range 2 0—4 5) In three cases the testicular/ovarian vein opened directly opposite the suprarenal vein and in four twice as far in In the remaining three cases, the testicular/ovarian vein did not open into the renal vein The mean distance from the site of opening to the vena cava was 5 09 cm (range 3—9)

The value for the PAH extraction by the left kidney without the contribution of blood from the tributaries can be calculated For this calculation, the apparent extraction and the RPF in the relevant kidney must be known as well as the quantity of plasma from the tributaries The blood flow from the left suprarenal vein was determined by Bucht (43) and found to be 2—5 ml/minute (mean in 5 healthy subjects 2 74 ml/minute) He estimated the blood flow from the ovarian/testicular vein to be approximately the same quantity

The corrected extraction E_c *i e*, that without admixture of blood from the tributaries can be calculated from the value for the PAH extraction (apparent extraction E_a) obtained in the usual way, according to the equation

$$E_c = E_a \frac{RPF}{RPF - b}$$

in which RPF is the renal plasma flow without correction for the tributaries and *b* is the quantity of plasma in the blood flowing in from the tributaries per minute In view of the difficulty of estimating the latter quantity, I calculated for a possible admixture of blood of both 5 and 10 ml/minute, *i e*, 3 and 6 ml/minute of plasma, respectively (Table VIII)

The RPF could be measured in only 7 of the 35 cases of chronic and acute pyelonephritis in which the PAH extraction by each of the kidneys was determined Consequently, the correction could not be made consistently in the comparison between right and left kidney Table VIII shows the influence of the correction on the PAH extraction value of the left kidney in the 7 cases in which it could be calculated The values were computed in relation to corresponding weighted clearance periods They do not therefore, correspond exactly to the mean values for PAH extraction and clearance respectively by the left kidney listed in Table X since the latter values were usually calculated as the mean of several periods The only reason for presenting the calculations was, in fact, to show the extent to which any admixture of blood from the tributaries may affect the extraction values It can be inferred from Table VIII that this correction changed the relevant values only inappreciably Consequently the difference does not alter the conclusion that as a rule the PAH extraction by the left kidney is smaller than that by the right

In my cases the catheter was advanced as far as possible in the left renal vein and then slowly withdrawn until a satisfactory flow was obtained on aspiration The position of the tip was checked by fluoroscopy The same procedure was used on the right side

In the present investigation I catheterized the right and left renal vein at the same session in 11 healthy volunteers and determined the PAH extraction by each kidney The results are listed in Table III from

Table VIII *Corrected* PAH *extraction by left kidney* (E_c L) *according to formula on p 62 for hypothetical influence of blood from the tributaries on the extraction value*

Case no	Right kidney C_{PAH} ml min	Right kidney E_a %	Right kidney RPF ml min	Left kidney C_{PAH} ml min	Left kidney E_a %	Left kidney RPF ml min	E_c for 3 ml/min	E_c for 6 ml min	$E_c - E_a$ for 3 ml min	$E_c - E_a$ for 6 ml min
4a	73	46.9	156	70	37.4	187	38.15	38.52	0.75	1.12
8a	167	78.3	213	119	58.9	202	60.08	60.67	1.18	1.77
12	175	61.5	285	213	72.5	295	73.23	73.95	0.73	1.45
13a	132	80.6	164	110	82.5	133	84.15	86.63	1.65	4.13
19a	50	69.1	72	36	69.6	52	73.78	78.65	4.18	9.05
20a	92	85.3	108	55	55.0	100	56.65	58.30	1.65	3.30
22a	51	31.9	160	17	18.3	93	18.85	19.58	0.55	1.28

For comparison the PAH clearance by both kidneys is given, as well as the PAH extraction (E_a) and RPF calculated in the conventional way The extraction is corrected *for a plasma flow from the tributaries of both 3 and 6 ml min*

The differences (% of renal extraction) between the hypothetical values and those calculated conventionally are listed in the last two columns

which it can be inferred that the extraction was the same bilaterally Since no significant difference was present between the right and left kidney in this respect it seems allowable to conclude that an admixture of blood from tributaries and from the caval vein does not play any essential role at any rate in healthy kidneys It is true that the normal series in question consisted of *young persons* and that the mean age in the series of patients was much higher It nevertheless seems unlikely that this age difference would have influenced the source of error which blood from the tributaries may imply

Ofstad (181) also found that the sampling error due to intermixture of non renal blood is small at the maximum of the same order of magnitude as the sum of the chemical analysis errors in measurements of E_{PAH} and not different on the two sides

Xylocaine® was used as a local anaesthetic when passing the catheter into the renal vein Since this preparation is excreted by non ionic diffusion it is highly improbable that it influenced the PAH extraction (76)

Despite all precautions I was not invariably able to avoid errors in determination of the renal extraction of PAH This is evident from the fact that in a few cases in which more than two determinations were made from *one side on the same occasion*, one value differed considerably from the others Obviously in such cases it cannot *be ruled out that a transient* change had occurred in the kidney's extraction ability It nevertheless seems more probable that one or more of the aforementioned sources of error had taken effect Which of *them* was responsible must remain an open question

If an individual value showed an *extremely* large deviation ($\geq$ 50 per cent of the mean value) it was however *considered to* be evident that the catheter tip had slipped so that the blood derived from the vena cava The deviating value was *then omitted* from the calculation of the mean value Such an omission was made *in* 5 cases (Table IX)

In case 128 with *stenosis* of the right main renal artery the mean value for PAH extraction by the left kidney was 5.8 per cent

Table IX E_{PAH} *values omitted*
All the E_{PAH} *values in the cases where the bracketed values were omitted from Table X*

Case no	Right	Left
2a	84 2	
	79 4	
	(23 1)	
14c	61 5	
	61 3	
	71 9	
	72 5	
	(6 7)	
	70 5	
	70 5	
	68 8	
84a		72 6
		74 0
		(7 4)
		77 6
		77 7
90a		12 1
		11 7
		(6 5)
		9 3
		35 3
117a	88 7	
	85 2	
	85 3	
	(12 3)	

This value was omitted from both the tables and the figures, since it was regarded as unquestionable that a technical error had been made The extraction of PAH by the right kidney was 71 0 per cent, but the creatinine clearance by this kidney was lower than that by the left kidney The osmolarity of the urine and its sodium concentration were the same bilaterally

In the tables, the cases in which one or several of the values for the renal extraction of PAH deviated from the mean by ≥ 25 per cent are marked with an asterisk beside the relevant value

Effect of Ureteric Catheterization on the Determinations

As far as separate determinations of the clearances are concerned, I departed from the principle of using only methods which did not require complete collection of the urine One of the reasons why this was regarded as permissible was that all clearances were determined during at least two consecutive periods Nevertheless, it cannot be denied that a risk of erroneous results existed

In the cases in the present series, it is evident that leakage of urine outside the ureteric catheter was an appreciable source of error in only a few cases Thus when the urine from the bladder was to be collected separately through an indwelling catheter, none was as a rule obtained However in three patients (cases 2, 5 136) the leakage amounted to 30, 19 and 35 per cent, respectively, of the total diuresis Their clearance values were therefore omitted from the tables and figures In the other 34 examinations in which clearance determinations were made, the leakage — if any — was so slight that it could not be measured

It has been suggested that the urine escaping into the bladder should be calculated in relation to the quantity of urine excreted per time unit by each kidney separately (51, 55 145 149 187 233) Such calculations are not however, to be recommended since it has been postulated that a difference is present between the urine which passes through the ureteric catheters and that which passes outside them with respect to composition and concentration (137) Thus no simple mathematical relation exists between the concentration of urine drained through the catheter and that escaping into the bladder Hoeg (102) also criticized the aforementioned

methods for calculating the leakage which he estimated at 1 7 per cent In addition, he pointed out that if the concentrations are identical such calculations cannot be made and if they differ slightly there is a wide range of uncertainty Haugen *et al* (94) too emphasized the importance of leakage in bilateral clearance determinations by means of ureteric catheterization Of 41 attempts at such determinations 16 were unsuccessful 6 of them on account of leakage

Fo the reasons given in the aforegoing I made no attempt to calculate how large a proportion of the urine collected in the bladder had leaked from the right and left ureteric catheter respectively

With respect to unilateral *versus* bilateral catheterization Dustan *et al* (65) stated that there is no disadvantage in catheterizing only one ureter with an occluding catheter and collecting the urine from the other via the bladder They found no support for the supposition that urine would leak undetected beside the ureteric catheter and mix with that from the other kidney collected in the bladder No were they of the opinion that catheterization has any effect on renal function

In my series unilateral ureteric catheterization was performed in only one patient (case 121) and the urine from the other side was collected from the bladder

The results of Prat & Kocvara (196) were in opposition to the view of Dustan *et al* (65) *i e* that ureteric catheterization has little if any effect on renal function The former authors stated that in most cases catheterization decreases the blood flow through the kidney and often causes a decrease in the glomerular filtration rate (GFR) as well They ascribed the latter to spasm of the vasa efferentia Diuresis usually increases as a result of diminished reabsorption of water as also found by Schück & Hradec (223) In another publication Prat & Kocvara (197) reported that changes in renal function occurred to approximately the same extent irrespective of whether catheterization was uni or bilateral They stated that even introduction of a catheter into the bladder could increase diuresis although only inappreciably

The experience of Larsen (145) and of Höeg (102) was in contrast to these observations They found that the clearance values determined on bladder urine during the control period before passage of the catheters were in almost every case higher than the sum of the clearances in the urine drained through both ureteric catheters

The effect of emotional factors on renal haemodynamics and function has also been studied by several authors *e g* by Brod & Sirota (32) who made experiments on dogs Hinkle *et al* (100) found in man that stimuli in the form of strong intimidation increased diuresis by 200—500 per cent in hydropaenic patients with an associated decrease in specific gravity and chloride concentration in the urine Miles & de Wardener (165) studied the emotional effect of catheterization in 10 normotensive and 10 hypertensive patients Increased diuresis was recorded in all of the latter it was sometimes large and was caused mainly by a raised excretion of Na and chlorides Nine of the 10 normotensive patients showed a slight increase in the urinary volume The authors stated that the increased electrolyte output was probably to be ascribed to decreased tubular reabsorption rather than to an associated increase in GFR They concluded that emotional factors may be res

ponsible for an abnormally high excretion of electrolytes

Wolf (271) found that pain decreased the renal blood flow, and explained this *observation on the basis of the view ex*pressed by Chasis *et al* (50), *i e*, that the renal plasma flow is regulated by the efferent glomerular arterioles This led Wolf to conclude that pain causes contraction of both the efferent and afferent arterioles with a resulting decrease in the flow of plasma through the kidney Because of the increase in the filtration fraction, the efferent vessels must contract to a greater extent — in other words, the renal plasma flow de*creases whereas the glomerular filtration* rate is maintained

The possibility cannot be disregarded that a purely mechanical irritation may elicit the aforementioned vasospasm Thus Hix (101) for example, stated that irritation of one ureter in the dog caused a decrease in renal plasma flow and glomerular filtration rate in this kidney He found sensory nerves *running from the ureter, which communi*cated reflexly with renal efferent constrictor fibres As mentioned previously, Haugen *et al* (94) were unable in 16 of 41 patients, to perform bilateral clearance determinations by means of ureteric catheterization In 5 of these cases, failure was due to the instrumentation causing *such discomfort to the* patient that the examination had to be dis*continued*

Spasm is apt to occur around the catheter Nevertheless according to Kiil (132), no reflexogenic disturbances occur even when there is negative pressure, despite adherence of the catheter tip to *the ureteric mucosa*

An additional factor of consequence for the urinary *volume* is the effect of the anti diuretic hormone (ADH) Introduction of a ureteric catheter may — via the central nervous system — reduce the production of ADH, and thereby decrease the reabsorption of water (176) Verney (258) showed *that* water diuresis does not recommence immediately after the decrease in diuresis caused by ADH, *but that 15—20 minutes* elapse before the ADH circulating in the blood is eliminated Consequently, the increased diuresis which I — like Prat & Kocvara (196) — sometimes observed during the first 5—15 minutes after passage of the catheter cannot be ascribed solely to suppression of *ADH* secretion Therefore, when determining the GFR and osmolarity *of catheter specimens, I always discarded* this first portion of urine De Wardener (263) described three types of emotional effect on diuresis In the first type, diuresis is decreased due to an enhanced production of ADH as a result of pain fear or similar reactions The second type of reaction is *osmotic diuresis Finally, the third type is* characterized by water diuresis caused by inhibition of ADH production

It is thus impossible, in the individual *case to decide which of the aforementioned* patterns — alone or in combination — plays the greatest role in altering the renal haemodynamics

In order to evaluate the function of each kidney properly it is important to have a basis of comparison in the form of a study of the combined activity of both kidneys made before the separate examination or in *some cases after it On technical grounds* this requirement could not be fulfilled in every case in the present series

In addition I tried as far as possible to eliminate the disturbing factors that may be elicited by catheterization One possibility

is to delay taking the specimens until 30—40 minutes after the catheters have been passed and the bladder emptied (196). This prolongation of the examination does however increase the risk of spasm around the catheters. For this reason, I generally confined myself to waiting until the urinary flow was relatively constant (usually 15—20 minutes). Every effort was made to prepare the patients psychologically and instrumentation was preceded by thorough local anaesthesia of the mucosa.

Figure 1 A and B illustrates that ureteric catheterization *per se* may constitute a source of error. It can be seen that, in some of my patients, a higher value was obtained for the renal concentration ability when the test was made without catheterization than that obtained for the better kidney at catheterization. This implies that the concentration ability had decreased, at any rate in the less involved kidney. It cannot be stated whether or not it was affected in the poorer kidney as well. This reduced renal concentration ability during ureteric catheterization is in agreement with the increased diuresis observed in this connexion by other authors cited in the aforegoing.

A lack of agreement between the renal concentration ability during ureteric catheterization and that without instrumentation is not necessarily to be ascribed to a reflex mechanism, to psychological factors or to pain caused by the instruments, since the two examinations were made at an interval of 3—7 *days*. *The state of the patient's* kidneys may therefore have changed, and the degree of dehydration may have *differed*. Figure 1 suggests that this source of error was of relatively little consequence, at any rate in the cases of chronic and acute pyelonephritis.

It is a common experience in serial clearance determinations — or in other investigations in which quantitative collection of urine is an essential factor — that consecutive double determinations in the same individual may show poor agreement. In view of the sources of error associated with ureteric catheterization it is evident that such a lack of conformity between values from different periods may be still more frequent when dealing with the function of one kidney only. This did in fact apply to some patients in my series.

It would perhaps have been easier to draw conclusions from the results if those with poor agreement between the periods had been omitted. In most cases the discrepancy is probably to be ascribed to technical errors or accidents, to the patient's subjective complaints or to other psychological factors. Nevertheless, it cannot be ruled out that a spontaneous change had occurred, *in e.g.* the glomerular filtration rate, diuresis and reabsorption of electrolytes during a short part of the investigation period. *For this* reason, I did not omit any values. However, since the mean values based on results with poor agreement between the periods are of little worth when conclusions are to be drawn, such deviations are noted *in the* tables. Thus, mean values in which the result of one or more determinations differed by ≥ 50 *per cent from the* mean are marked with a circle (o). This limit was ≥ 25 per *cent for* E_{PAH} *and the* relevant value is marked with an asterisk (*cf.* page 64). When making determinations on catheter specimens it does not seem permissible to omit a single value as unquestionably due to a technical *error*, as was considered justified with respect to values for the renal extraction of PAH (Table IX).

ponsible for an abnormally high excretion of electrolytes

Wolf (271) found that pain decreased the renal blood flow and explained this observation on the basis of the view expressed by Chasis *et al* (50) *i e* that the renal plasma flow is regulated by the efferent glomerular arterioles This led Wolf to conclude that pain causes contraction of both the efferent and afferent arterioles with a resulting decrease in the flow of plasma through the kidney Because of the increase in the filtration fraction the efferent vessels must contract to a greater extent — *in other words* the renal plasma flow decreases whereas the glomerular filtration rate is maintained

The possibility cannot be disregarded that a purely mechanical irritation may elicit the aforementioned vasospasm Thus Hix (101) for example stated that irritation of one ureter in the dog caused a decrease in renal plasma flow and glomerular filtration rate in this kidney He found sensory nerves running from the ureter which communicated reflexly with renal efferent constrictor fibres As mentioned previously Haugen *et al* (94) were unable in 16 of 41 patients to perform bilateral clearance determinations by means of ureteric catheterization In 5 of these cases failure was due to the instrumentation causing such discomfort to the patient that the examination had to be discontinued

Spasm is apt to occur around the catheter Nevertheless according to Kiil (132) no reflexogenic disturbances occur even when there is negative pressure despite adherence of the catheter tip to the ureteric mucosa

An additional factor of consequence for the urinary volume is the effect of the antidiuretic hormone (ADH) Introduction of a ureteric catheter may — via the central nervous system — reduce the production of ADH and thereby decrease the reabsorption of water (176) Verney (258) showed that water diuresis does not recommence immediately after the decrease in diuresis caused by ADH but that 15—20 minutes elapse before the ADH circulating in the blood is eliminated Consequently the increased diuresis which I — like Prat & Kocvara (196) — sometimes observed during the first 5—15 minutes after passage of the catheter cannot be ascribed solely to suppression of ADH secretion Therefore when determining the GFR and osmolarity of catheter specimens I always discarded this first portion of urine De Wardener (263) described three types of emotional effect on diuresis In the first type diuresis is decreased due to an enhanced production of ADH as a result of pain fear or similar reactions The second type of reaction is osmotic diuresis Finally the third type is characterized by water diuresis caused by inhibition of ADH production

It is thus impossible in the individual case to decide which of the aforementioned patterns — alone or in combination — plays the greatest role in altering the renal haemodynamics

In order to evaluate the function of each kidney properly it is important to have a basis of comparison in the form of a study of the combined activity of both kidneys made before the separate examination or in some cases after it On technical grounds this requirement could not be fulfilled in every case in the present series

In addition I tried as far as possible to eliminate the disturbing factors that may be elicited by catheterization One possibility

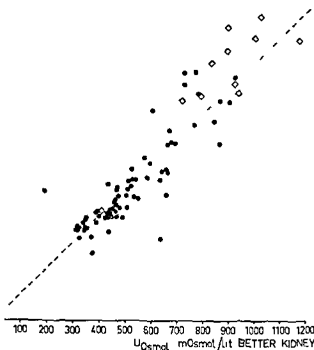
is to delay taking the specimens until 30—40 minutes after the catheters have been passed and the bladder emptied (196) This prolongation of the examination does however increase the risk of spasm around the catheters For this reason I generally confined myself to waiting until the urinary flow was relatively constant (usually 15—20 minutes) Every effort was made to prepare the patients psychologically and instrumentation was preceded by thorough local anaesthesia of the mucosa

Figure 1 A and B illustrates that ureteric catheterization *per se* may constitute a source of error It can be seen that in some of my patients a higher value was obtained for the renal concentration ability when the test was made without catheterization than that obtained for the better kidney at catheterization This implies that the concentration ability had decreased at any rate in the less involved kidney It cannot be stated whether or not it was affected in the poorer kidney as well This reduced renal concentration ability during ureteric catheterization is in agreement with the increased diuresis observed in this connexion by other authors cited in the aforegoing

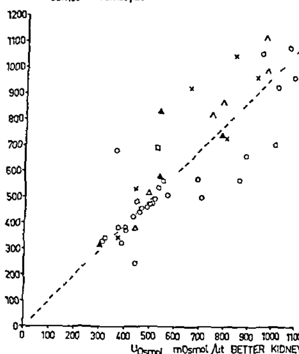
A lack of agreement between the renal concentration ability during ureteric catheterization and that without instrumentation is not necessarily to be ascribed to a reflex mechanism to psychological factors or to pain caused by the instruments since the two examinations were made at an interval of 3—7 days The state of the patient s kidneys may therefore have changed and the degree of dehydration may have differed Figure 1 suggests that this source of error was of relatively little consequence at any rate in the cases of chronic and acute pyelonephritis

It is a common experience in serial clearance determinations — or in other investigations in which quantitative collection of urine is an essential factor — that consecutive double determinations in the same individual may show poor agreement In view of the sources of error associated with ureteric catheterization it is evident that such a lack of conformity between values from different periods may be still more frequent when dealing with the function of one kidney only This did in fact apply to some patients in my series

It would perhaps have been easier to draw conclusions from the results if those with poor agreement between the periods had been omitted In most cases the discrepancy is probably to be ascribed to technical errors or accidents to the patient s subjective complaints or to other psychological factors Nevertheless it cannot be ruled out that a spontaneous change had occurred *in e g* the glomerular filtration rate diuresis and reabsorption of electrolytes during a short part of the investigation period For this reason I did not omit any values However since the mean values based on results with poor agreement between the periods are of little worth when conclusions are to be drawn such deviations are noted in the tables Thus mean values in which the result of one or more determinations differed by ≥ 50 per cent from the mean are marked with a circle (o) This limit was ≥ 25 per cent for E_{PAH} and the relevant value is marked with an asterisk (*cf* page 64) When making determinations on catheter specimens it does not seem permissible to omit a single value as unquestionably due to a technical error as was considered justified with respect to values for the renal extraction of PAH (Table IX)



A Chronic and acute non obstructive pyelonephritis (groups A1 A2)



B Miscellaneous renal diseases (groups B1—B5)

Fig 1 Concentration ability (mOsmol/lit) of both kidneys (total) without catheterization (ordinate) and concentration ability of the better kidney (abscissa) — The broken line represents symmetric values For symbols see p 39

I did not investigate whether, in my cases, ureteric catheterization had influenced other renal functions than the concentration ability Obviously, it cannot be excluded that the renal plasma flow, clearances PAH extraction and glomerular filtration rate were also affected Nor does my series allow any conclusions about whether or not the functional changes elicited by catheterization in several cases were symmetric

CHAPTER IX

RESULTS

The results of the tests in which the function of *each kidney* was studied separately are given in Table X.

In most of the cases in groups A1 A2 B1 and B2 in which asymmetric renal function was observed all the parameters studied were asymmetric in the same direction *i e* one kidney functioned better than the other in every such respect This is demonstrated in Tables XI and XII

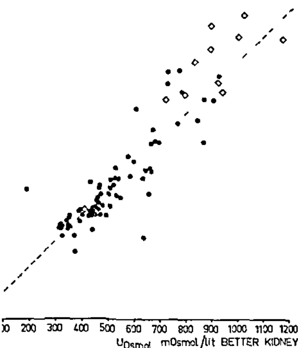
In the cases of stenosis of the main renal artery with arterial hypertension (group B3) the pattern differed from that in the aforementioned groups as can be inferred from Tables X XI and XII This pattern will be discussed in Chapter X (pp 114—116) As far as groups B4 and B5 are concerned it can be seen in Tables XI and XII that in the great majority of cases there was symmetry between the two kidneys as regards roentgenologic features bacteriologic culture of the urine tenderness to palpation over the loins and subjective complaints whereas more divergent results were noted in other tests

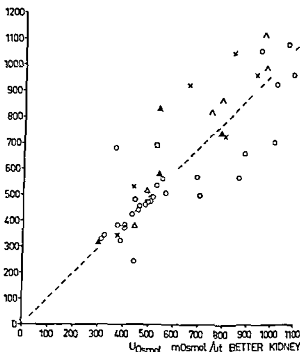
In the tables poorer function of the right kidney is indicated by an arrow pointing upwards (↑) and poorer function of the left kidney by an arrow pointing downwards (↓) The sign = denotes that the difference between the kidneys did not exceed the limits for symmetry of the various parameters given in Chapter I The sign [=] denotes that there was not only no difference between the relevant function of the two kidneys but also that both values lay within the normal range

An analysis of Table XI shows that in certain cases the asymmetry of one or several individual functions was in the opposite direction to that of the other functions Table XII therefore shows the number of investigations in which the parameters tested were symmetric those in which they were in agreement with the majority of the other bilateral investigations in being asymmetric and those in which the asymmetry was not consistent Finally it contains a group of cases denoted as indecisive since the comparative studies were either too few or gave too indecisive results to permit evaluation of which kidney had the better function

Since the PAH clearance is of little value in determinations in diseased kidneys these results have been omitted from Tables XI and XII The endogenous creatinine and inulin clearances are expressions of the same function they have therefore been counted as one test when both arrows pointed in the same direction In the few cases in which the arrows representing creatinine and inulin clearance pointed in the opposite direction that of the former has been taken as decisive since for practical reasons the creatinine clearance was determined in more cases than the inulin clearance

A few of the investigations in which the asymmetry of various parameters of renal function was inconsistent merit brief comment An example of a case in which the renal concentration ability differed from the other tests is the following





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B Miscellaneous renal diseases (groups B1—B5)

Fig 1 Concentration ability (mOsmol/lit) of both kidneys (total) without catheterization (ordinate) and concentration ability of the better kidney (abscissa) — The broken line represents symmetric values For symbols see p 39

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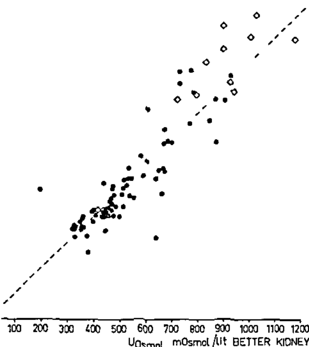
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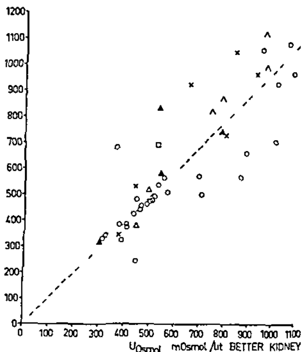
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Table Xa. *Investigations of each kidney*

1	5				6			7			
Case no	UCr (mEq/lit)				UpH			EPAH ( )			
	R	L	Diff		R	L	Diff	R	L	Diff	
GROUP A1 Chronic non-obstructive pyelonephritis (≥ 3 cardinal criteria)											
1	—		—	—	—	—	—	8_2	74 4	− 7 8	9
_a		—	—	—	5 10	4 98	−0 12	81 8	64 1	−17 7	—
b	—	—	—	—	—	—	—	—	—	—	—
3	80	50	−30	38	—	—	—	57 8	64 7	+ 6 4	10*
4a			—	—	5 47	5 44	−0 03	47 8	39 2	− 8 6	18
4b		—	—	—	—	—	—	74 7	73 6	− 1 1	1
5	—		—	—	—	—	—	—	—	—	—
6b	—		—	—	—	—	—	—	—	—	—
6c	70	55	−15	21	—	—	—	75 6	60 1	−15 5	21
7a			—	—	—	—	—	89 4	71 0	−18 4	21
7b	70	60	−10	14	—	—	—	—	—	—	—
8a	—		—	—	5 00	4 96	−0 04	79 0	59 7	−19 3	74
8b		—	—	—	—	—	—	63 0	54 6	− 8 4	13
9a			—	—	6 60	6 65	+0 05	53 5	44 8	− 8 7	16
9b	65	55	−10	15	—	—	—	8_8	74 0	− 8 8	11
10	—			—	5 10	5 09	−0 01	86 6	82 6	− 4 0	5
11		—	—	—	—	—	—	17 5	14 7	− 2 8	16
1				—	5 77	5 60	−0 17	60 7	72.5	+11 8	16
13a				—	5 1	5 1	0	81 6	8_3	+ 0 7	1
13b	—	—	—	—	—	—	—	90 4	88 6	− 1 8	2
14a	—			—	—	—	—	49 0	38 2	−10 8	22
14b	—			—	—	—	—	—	—	—	—
14c			—	—	—	—	—	68 1	63 2	− 4 9	7
15		—	—	—	—	—	—	75 5	64 3	−11.2	15
16	50	80	+30	38	—	—	—	53 6	87 7	+34 1	39
17				—	—	—	—	76 4	37 7	−38 7	51
18				—	—	—	—	34 7	19 6	15 1	44
19a			—	—	—	—	—	69 4	70 0	+ 0 6	1
19b			—	—	—	—	—	69 4	69 2	− 0 2	0
20a				—	—	—	—	86 2	59 7	−_6 5	31
20b	—				—	—	—	—	—	—	—
1											
2 a				—	—	—	—	—	—	—	—
2 b	10	8	2	70	—	—	—	36 0	21 9	−14 1	39*
23	70	110	+40	36	—	—	—	65.2	58 9	− 6 3	10
24	—		—	—	—	—	—	—	—	—	—
5a	—		—		—	—	—	—	—	—	—
25b	45	70	+ 5	36	—	—	—	—	—	—	—
26b					—	—	—	—	—	—	—
27			—		—	—	—	—	—	—	—
8c			—	—	—	—	—	92.4	87.2	− 5.2	6
79c			—		—	—	—	70 4	44 5	25 9	37
30b					—	—	—	79 8	71 8	− 8 0	10
31					—	—	—	83 7	79 8	− 3 9	5
				—		—	—	95 9	96 0	+ 0 1	0
GROUP A2 Acute and suspected chronic non-obstructive pyelonephritis (< 3 cardinal criteria)											
70			—		—	—	—	85 8	89 5	+ 3 7	4
71			—		5 10	5 4	+0 14	86 1	83 3	− 2.8	3
72				—	—	—	—	87 3	88 2	+ 0 9	1

Col mn 1
a, b c and d de ote e aminations on different occasions in the same pati t.

Co mn 2
The test was made w thout dehydration or adm istration of Pi ress n® when the relev nt value is marked by

Column 7
When one or more of the E_{PAH} values de iated from the me n by ≥ _5 ,, the rele ant value is marked w th an a terisk The cases in whi h the values were om tted are fo nd in Table IX

Foot-not s
See column 16

Table Xa *Investigations of each kidney*

1	2				3				4		
Case no	Max conc ability (mOsmol/lit)				U_{Na} (mEq/lit)				U_K (mEq/lit)		
	R	L	Diff	%	R	L	Diff	%	R	L	Diff
	GROUP A1 Chronic non obstructive pyelonephritis (≥ 3 cardinal criteria)										
1	535	505	− 30	6	80	58	−22	28	—	—	—
2a	409	426	+ 17	4	—	—	—	—	—	—	—
2b	471	500	+ 29	6	—	—	—	—	—	—	—
3	545	405	−140	26	146	130	−16	11	—	—	—
4a	326	230	− 96	29	—	—	—	—	—	—	—
4b	430	410	− 20	5	128	121	− 7	5	—	—	—
5	339	271	− 68	20	—	—	—	—	—	—	—
6b	460	405	− 55	12	—	—	—	—	—	—	—
6c	465	440	− 25	5	78	92	+14	15	—	—	—
7a	425	488	+ 63	13	—	—	—	—	—	—	—
7b	512	523	+ 11	2	138	148	+10	7	—	—	—
8a	430	414	− 16	4	—	—	—	—	—	—	—
8b	—	—	—	—	—	—	—	—	—	—	
9a	313	300	− 13	4	—	—	—	—	—	—	—
9b	507	491	− 16	3		—	—	—	—	—	—
10	627	763	+136	18	—	—	—	—			
11	370	343	− 27	7	—	—	—	—	—	—	—
12	265	376	+111	30	—	—	—	—	—	—	—
13a	500	437	− 63	13		—	—	—	—	—	—
13b	572	577	+ 5	1	360	348	−12	3	—		—
14a	505	495	− 10	2	—		—	—	—	—	
14b	465	460	− 5	1			—	—	—	—	—
14c	560	655	+ 95	15	61	50	−11	18	—	—	—
15	441	379	− 62	14	—	—	—	—	—		
16	377	477	+100	21	106	110	+ 4	4	—	—	—
17	355	395	+ 40	10	—		—		—	—	
18	398	368	− 30	8	—	—	—	—	—		—
19a	306	390	+ 84	22		—		—	—	—	
19b	360	465	+105	23	18	43	+25	58			
20a	438	286	−152	35	—		—	—	—	—	
20b	655	330	−325	50	—	—	—	—			
21	287	218	− 69	24	75	72	− 3	4	22 6	14 2	8 4
22a	318	229	− 89	28	—			—	—	—	
22b	—	258	—		25	33	+ 8	24	—		
23	670	915	+245	27	198	228	+30	13			—
24	318	355	+ 37	10		—	—	—	—	—	
25a	265	440	+175	40	—						
25b	393	455	+ 62	14	118	120	+ 2	[illegible]		—	
26b	333	433	+100	23			—	—	—	—	
27	777	717	− 60	8	—	—	—	—		—	
28c	—	—	—					—	—		—
29c	—	—	—	—				—	—		
30b	—	—	—	—	—	—	—	—	—	—	
31	—	—	—								
	GROUP A2 Acute and suspected chronic non obstructive pyelonephritis (< 3 cardinal criteria)										
70	680	883	+203	23	—	—	—	—	—		
71	671	714	+ 43	6	—				—		
72	965	1013	+ 48	5	220	240	+ 0	8		—	—

The d fferences between the r ght and left k dney are g ven both in absolute figures (where + denotes that the right k dney has the h gher value) and in of the value for the better k dney (The d fferences in C_{PAH} have been om tted)

When one or more of the values obta ned at ureter c catheterizat on deviated from the mean by ≥ 50 % the relevant value is marked by

Symbols

- 0 Noth ng pathologic observed or answer to quest on negat ve
- \+ Patholog c finding or answer to question pos t ve
- (+) Doubtful pos t ve finding or doubtful pos t ve answer
- — Not invest gated or questioned

Table Xa (continued)

1	11				12		13		14		15	
Case no	Cl_{in} (ml min)				Urine culture (bact/ml)		Roentgenol exam		Loin tenderness to palp		History of loin pain	
	R	L	Diff		R	L	R	L	R	L	R	L
GROUP A1 Chronic non-obstructive pyelonephritis (≧ 3 cardinal criteria)												
1	–	–	–	–	2 mill.	0	(+)	+	0	0	+	0
2a	–	–	–		–	–	+	+	0	0	(+)	(+)
2b	–	–	–	–		0	+	(+)	0	0	0	0
3	–	–	–	–	0	80 000	0	+	0	0	+	0
4a	18	14	−4	22	–	–	+	+	0	0	+	+
4b	–	–	–	–	0	300 000	+	+	0	0	+	+
5	–	–			1 mill.	300 000	(+)	+	0	0	0	0
6b	–	–	–	–	3 000	230 000	(+)	+	+	+	0	0
6c	–	–	–		0	0	(+)	+	0	0	0	0
7a	–				0	0	0	0	0	0	0	0
7b		–	–	–	5 000	5 000	0	0	0	0	0	0
8	36	31	−5	14	–		0	0	0	0	0	0
8b		–				–	0	+	0	0	0	0
9a	13	8	5	38	50 000	50 000	0	+	0	0	0	0
9b	–	–	–	–	0	0	(+)	+	0	0	0	0
10		–	–		–	–	0	0	0	0	0	0
11	5	7	+2	[illegible]	–	–	0	0	+	+	+	+
12	41	55	+14	[illegible]	5 000	5 000	+	(+)	0	0	–	–
13a	38	34	4	10			(+)	+	0	0	0	0
13b		–			0	0	(+)	+	0	0	0	0
14a		–	–	–	50 000	400 000	(+)	+	0	0		–
14b			–				(+)	+	0	0	–	–
14c	–	–	–		0	0	+	+	0	0	+	0
15		–		–	–	–	0	+	0	+	+	+
16		–		–	>100 000	0	+	(+)	0	0	–	–
17		–	–		1 500	80 000	(+)	+	0	0	+	(+)
18			–		1 100	0	+	0	+	+	–	–
19a	15	11	4	27			+	(+)	0	0	0	0
19b				–	100	100	+	(+)	0	0	0	0
20a	[illegible]	19	10	34	50 000	50 000	(+)	+	0	+	0	+
20b	–	–	–		400 000	140 000	(+)	+	0	+	0	0
21		–	–		0	5 000	(+)	+	(+)	0	–	–
22a	1	5	−7	58			+	+	0	0	0	+
22b		–	–	–	–	0	(+)	+	+	0	0	0
23		–		–	50	0	+	0	0	0	0	0
24				–	50 000	800 000	(+)	+	+	0	0	0
25a		–		–	>100 000	0	0	0	0	0	+	0
25b		–	–		15 000	6 000	+	(+)	0	0	0	0
26b		–	–	–		–	(+)	0	0	(+)	0	0
27			–	–	0	0	0	+	0	+	0	+
28c			–		0	–	+	+	0	0	+	0
29c				–	50 000	>100 000	+	(+)	+	0	+	0
30b			–			–	+	+	0	0	0	0
31				–		–	0	0	+	0	0	0
GROUP A2 Acute and suspected chronic non-obstructive pyelonephritis (< 3 cardinal criteria)												
70			–		0	0	0	0	0	0	+	0
71	1–	132	+10	8	–	–	0	0	0	0	–	
72				–	135	0	0	0	0	0	+	0

16

E_D (%)			
R	L	Diff	
43.–	4..1	−1.1	3

* Am chlor load

U_{Cl} (mEq/lit)			
R	L	Diff	
78	71	−7	9

E_D (%)			
R	L	Diff	
* 26.5	16.5	−10.0	38
37.8	31.1	−6.7	18

E_D (%)			
R	L	Diff	
43.6	2..8	25.8	53

Table Xa (continued)

1	8				9		10		
Case no	RPF (ml/min)				CPAH (ml/min)		CCr (ml/min)		
	R	L	Diff	%	R	L	R	L	Diff
GROUP A1 Chronic non-obstructive pyelonephritis (≥ 3 cardinal criteria)									
1	—	—	—	—	—	—	—	—	—
2a	—	—	—	—	—	—	—	—	—
2b	—	—	—	—	—	—	—	—	—
3	—	—	—	—	—	—	—	—	—
4a	156	187	+31	17	73	70	17	15	− 2
4b	—	—	—	—	—	—	—	—	—
5	—	—	—	—	—	—	—	—	—
6b	—	—	—	—	—	—	—	—	—
6c	—	—	—	—	—	—	—	—	—
7a	—	—	—	—	—	—	—	—	—
7b	—	—	—	—	—	—	—	—	—
8a	213	202	−11	5	167	119	31	22	− 9
8b	—	—	—	—	—	—	—	—	—
9a	—	—	—	—	43	27	21	13	8
9b	—	—	—	—	—	—	—	—	—
10	—	—	—	—	—	—	—	—	
11	—	—	—	—	17	21	5	6	+ 1
12	285	295	+10	3	162	213	28	37	+ 9
13a	164	133	−31	19	130	107	26	20	6
13b	—	—	—	—	—	—	—	—	
14a	—	—	—	—	—	—	—	—	—
14b	—	—	—	—	—	—	—	—	—
14c	—	—	—	—	—	—	—		
15	—	—	—	—	—	—	—		—
16	—	—	—	—	—	—	—	—	—
17	—	—	—	—	—	—	—	—	—
18	—	—	—	—	—	—	—	—	—
19a	72	52	−20	28	50	36	20	15	5
19b	—	—	—	—	—	—	—	—	
20a	108	100	− 8	7	92	55	26	12	14
20b	—	—	—	—	—	—	—		
21	—	—	—	—	75	153	24	40	+16
22a	160	93	−67	42	51	17	16	6	−10
22b	—	—	—	—	—	—	—		—
23	—	—	—	—	—	—		—	
24	—	—	—	—	—	—	—		—
25a	—	—	—	—	—	—	—	—	
25b	—	—	—	—	—	—	—		
26b	—	—	—	—	—	—	—	—	
27	—	—	—	—	—	—	—	—	
28c	—	—	—	—	—	—	—	—	—
29c	—	—	—	—	—	—	—		
30b	—	—	—	—	—	—	—		
31	—	—	—	—	—	—			
GROUP A2 Acute and suspected chronic non obstructive pyelonephritis (< 3 cardinal criteria									
70	—	—	—	—	451	433	61	55	− 6
71	—	—	—	—	—	—	—	—	—
72	—	—	—	—					

Table Xa (continued)

1	5				6			7		8		9		10	
Case no	Ucr (mEq/lit)				UpH			Urine culture (bact/ml)		Roentgenol exam		Loin tenderness to palp		History of loin pain	
	R	L	Diff		R	L	Diff	R	L	R	L	R	L	R	L
	GROUP A1 Chronic non-obstructive pyelonephritis (≥ 3 cardinal criteria)														
3	550	650	+10	15		–	–	1.2 mill.	600 000	+	+	0	+	0	+
33	–	–	–	–	–	–	–	0	0	0	0	0	0	–	–
34	–	–	–	–		–	–	70	300 000	(+)	+	+	+	0	+
35	60	70	+10	14	–			0	0	–	–	0	0	–	–
36		–	–	–		–	–	–	–	0	0	0	0	+	(+)
37	110	1 0	+10	8	–	–	–	0	0	0	0	0	0	–	–
38	75	50	–25	33	–	–	–	0	200 000	(+)	+	0	+	0	+
39				–			–	0	0	+	(+)	0	0	0	0
40	–	–	–	–	–	–	–	>100 000	0	+	(+)	+	0	+	0
41	–	–	–			–	–	60 000	.00 000	+	+	0	0	0	0
4	93	90	3	3	7 76	7 49	–0 27	–	–	0	0	0	0	–	–
43		–	–				–	70	0	0	0	+	0	+	0
44	80	90	+10	11	5 95	5 68	–0 7	0	0	+	(+)	0	0	–	–
45	–			–		–		–	0	+	0	0	0	+	0
46		–	–			–	–	0	0	(+)	(+)	0	+	+	+
47		–	–	–	–	–		1 mill.	3 mill	0	0	0	0	0	+
48	[illegible]	1 5	+ 3	2	6 79	6 05	–0 4	0	0	0	0	0	0	(+)	+
49	–		–		6 67	6 17	–0 50	0	0	0	0	+	0	+	0
50	90	50	–40	44	–	–		–	–	(+)	(+)	0	0	–	–
51	105	54	51	49	5 85	6 94	+1 09	0	25 000	(+)	+	0	0	0	+
5	130	110	– 0	15	5 1	6 1	+1 00	0	0	(+)	+	0	0	–	–
53	140	130	–10	7	7 79	7 69	–0 10	0	0	0	0	+	+	+	0
54		–	–	–				50 000	30 000	(+)	+	0	0	–	–
55	100	140	+40	79		–		0	0	0	+	+	+	+	0
56	88	78	10	11	5 75	5 75	0	4 mill	4 mill	(+)	(+)	+	+	0	+
57	100	100	0	0				0	1 300	0	0	+	+	–	–
58	0 7	0 8	+0 1	13	7.26	7 87	+0 61	40 mill	900	+	(+)	0	0	–	–
59				–	–		–	5 000	0	+	+	+	0	+	(+)
60					–			0	0	(+)	+	+	0	+	0
61		–	–					5 000	50 000	+	(+)	(+)	0	(+)	0
6		–	–			–	–	0	60 000	0	(+)	0	+	0	+
63		–	–	–		–	–	250 000	300 000	+	+	0	0	–	–
64		–	–	–	–	–		0	0	0	+	0	0	0	0
65		–	–	–	–	–	–	0	4 000	+	+	+	0	+	0
66		–	–	–											
67	150	160	10	6	–	–	–	600 000	1 mill	0	+	0	0	–	–
68		–	–	–		–	–	0	0	0	0	0	+	+	+
69	1 3	137	1	9		–		0	0	0	0	0	0	(+)	0
									–	0	0	0	0	0	0
	GROUP A2 Acute and suspected chronic non-obstructive pyelonephritis (< 3 cardinal criteria)														
73		–		–											
74				–	–		–	1 000	400 000	0	0	0	0	0	0
75	90	60	30	33	–		–	0	0	0	0	0	0	–	–
76			–		–		–	0	0	0	0	0	0	0	0
77			–		–	–	–	0	0	0	0	0	0	0	(+)
78	80	60	–0	[illegible]		–	–	0	0	0	+	0	0	0	0
79	135	150	+15	10		–	–	0	0	0	+	0	0	0	0
80	110	90	0	18			–	0	0	+	0	0	0	0	0
								0	0	0	0	0	0	–	–

Table Xa (continued)

1	2				3				4		
Case no	Max conc ability (mOsmol/lit)				U_{Na} (mEq/lit)				U_K (mEq/lit		
	R	L	Diff	/	R	L	Diff		R	L	Diff
GROUP A1 Chronic non obstructive pyelonephritis (≥ 3 cardinal criteria)											
32	438	450	+ 12	3	128	123	5	4	—	—	
33	702	834	+131	16	221	246	+25	10	—	—	
34	663	603	− 60	9	107	104	− 3	3	—	—	
35	340	351	+ 11	3	88	84	4	5	—	—	
36	596	599	+ 3	1	—	—	—	—	—	—	
37	663	673	+ 10	1	189	200	+11	6	—	—	
38	517	410	−107	21	100	63	−37	37	—	—	
39	350	300	− 50	14	13	13	0	0	—	—	
40	305	250	− 55	18	—	—	—	—	—	—	
41	560	643	+ 83	13	—	—	—	—	—	—	
42	575	690	+115	17	130	132	+ 2	2	—	—	—
43	352	463	+111	24	99	144	+45	31	—	—	—
44	233	318	+ 85	27	86	104	+18	17	—	—	—
45	605	722	+117	16	—	—	—	—	—	—	
46	323	250	− 73	23	—	—	—	—	—	—	—
47	463	470	+ 7	1	—	—	—	—	—	—	—
48	7[illegible]0	670	− 50	7	177	183	+ 6	3	—	—	—
49	735	765	+ 30	4	174	192	+18	9	—	—	—
50	635	322	−313	49	111	71	−40	36	—	—	—
51	505	348	−157	31	—	—	—	—	—	—	—
52	595	440	−155	26	140	142	+ 2	1	—	—	—
53	705	858	+153	18	152	168	+16	10		—	—
54	380	440	+ 60	14	80	80	0	0	—	—	—
55	690	7[illegible]0	+ 30	4	166	207	+41	20	—	—	—
56	452	405	− 47	10	88	84	− 4	5	—	—	—
57	665	653	− 12	2	168	168	0	0	—	—	—
58	555	858	+303	35	195	240	+45	19	—	—	—
59	345	352	+ 7	2	—	—	—	—	27	23	−4
60	460	394	− 66	14	99	98	− 1	1			
61	393	364	− 29	7	—	—	—	—	—	—	—
62	630	395	−235	37	—	—	—	—		—	—
63	460	578	+118	20	—	—	—	—	—	—	—
64	525	240	−285	54	—	—	—	—	—	—	—
65	188	163	− 25	13	—	—	—	—	—	—	—
66	530	485	− 45	8	—	—	—	—	—	—	—
67	660	622	− 38	6	100	88	−12	12	—	—	—
68	893	863	− 30	3	—	—	—			—	—
69	340	349	+ 9	3	42	47	+ 5	11			
GROUP A2 Acute and suspected chronic non-obstructive pyelonephritis (< 3 cardinal criteria											
73	855	915	+ 60	7	201	[illegible]04	+ 3	1	—	—	—
74	8[illegible]0	820	± 0	0	—	—		—	—	—	—
75	785	700	− 85	11	231	198	− 33	14	—		
76	765	930	+165	18	137	103	− 34	25	[illegible]0	27	− 7
77	882	793	− 89	10	175	175	± 0	0	—	—	—
78	404	394	− 10	2	76	90	+ 14	16	—	—	—
79	775	990	+215	22	258	282	+ 24	9			
80	1161	1153	− 8	1	285	270	− 15	5			

Table Xb *Investigations of each kidney*
Symbols as in Table Xa

1	5				6			7			
Case no	U_{Cr} (mEq/lit)				U_{pH}			E_{PAH} (%)			
	R	L	Diff		R	L	Diff	R	L	Diff	
GROUP B1 Chronic pyelonephritis with signs of urinary tract obstruction (≥ 3 cardinal criteria)											
81		–	–	–		–	–	73.0	69.3	– 5.7	8
8 a	–	–	–	–	–	–	–	–	–	–	–
8 b		–	–	–		–	–	–	–	–	–
8_c	50	45	5	10	7.4	7.7	+ 0.3	–	–	–	–
8 d	0	30	+10	33	6_2	6.31	+0.09	9.7	36.8	+ 7.1	74
83b		–	–	–	–	–	–	63.9	75.5	– 5.6	7
84a		–	–			–	–	56.0	55	– 0.8	1
84b	–	–	–	–	–	–	–	64.4	71.4	+ 7.0	10
85			–	–				–	–	–	–
86b		–	–	–	–	–		72.9	70.3	– _6	4
86c		–	–	–			–	26.9	27.7	+ 0.8	3
87	–	–	–	–			–	94.0	96.1	+ 2.1	2
88		–		–	–	–		–	–	–	–
89		–	–	–	–	–		87.0	39.7	–47.3	54
4c	90	85	– 5	6		–	–				
90a	–	–	–	–	–	–		34.8	17.1	–17.7	51
90b		–	–	–		–	–	49.8	4_8	– 7.0	34
91			–	–				88.1	9_	–78.9	90
9	135	65	70	52	6.67	7.8	+0.61	–	–	–	–
93			–	–		–		–	–	–	–
GROUP B2 Renal disease (except pyelonephritis) with unilateral or mainly asymmetric involvement											
106		–	–		–		–	6 9	57.9	– 5.0	8
107		–	–	–	4.75	5.15	+0.40	86.0	1_0	74.0	86
108			–			–		65.4	73.?	+ 7.8	11
109							–	87.6	83.1	4.5	5
110			–	–	–	–	–	59.7	68.1	+ 8.4	1_
111			–	–		–		89.7	86.3	– 3.4	4
11	31	39	+ 8	21			–	96.7	31.5	–65._	67
GROUP B3 Arterial hypertension with stenosis or aneurysm of the main renal artery											
117a				–		–	–	86.4	83.2	– 3.?	4
117b	8	16	+ 8	40		–	–	73.4	74.9	+ 1.5	2
118				–		–		76.5	8_8	+ 6.1	8
119		–			–		–	99.1	94.1	– 5.0	5
1 0			–				–	8 9	8 1	0.8	1
121					–		–	89.0	84.0	– 5.0	6
1	40	0	0	40		–		91.0	90.9	– 0.1	0
123				–		–	–	89.1	83.4	– 5.7	6
124						–	–	94.7	95.8	+ 1.1	1
1 5							–	90.7	88.0	– 2.7	3
1 6	–		–	–		–	–	90._	87.4	2.8	3
1 7		–	–	–		–	–	80.9	84.8	+ 3.9	5
128			–	–	–	–	–	–	–	–	–
1 9			–	–	–	–	–	88.1	85.7	– 2.4	3
GROUP B4 Renal disease with expected homogeneous involvement of both kidneys											
13				–			–	91.7	94._	– 3.5	4
133			–	–	–		–	87.7	84.6	– 3.1	4
134				–	–	–	–	75.6	66.1	9.5	13
136				–	5.7	5.7	0	74.6	7_9	– 1.7	_
137					–		–	8_9	86.9	+ 4.0	5
138a			–		4.85	4.90	+0.05	18.8	77.3	+58.5	76
139			–		–		–	88.1	89.5	+ 1.4	2
140				–	–		–	90._	90.0	– 0._	0
141			–	–		–	–	83.7	8 5	– 1.6	2
14					–	–	–	93.7	7	18 1	19
143				–	–	–	–	85.9	76.6	– 9.3	11
GROUP B5 Patients with symptoms from the lower urinary tract in whom involvement of the kidneys could not be ruled out											
148			–	–	–		–	87	90.8	+ 3.6	4
149	–		–	–	5.35	4_0	–0.05	81.7	88.3	+ 6.6	7
150		–		–	–	–		87.5	88.3	+ 0.8	1

Table Xb *Investigations of each kidney*
Symbols as in Tabl Xa

1	2				3				4			
Case no	Max conc ability (mOsmol/lit)				U_{Na} (mEq/lit)				U_K (mEq/lit)			
	R	L	Diff	/	R	L	Diff		R	L	Diff	
	GROUP B1 Chronic pyelonephritis with signs of urinary tract obstruction (≥ 3 cardinal criteria)											
81	452	434	− 18	4	–	–	–	–	–	–	–	–
82a	302	307	+ 5	2	–	–	–		–	–	–	–
82b	397	390	− 7	2	–	–	–		–	–	–	–
82c	480	423	− 57	12	–	–	–	–	–	–	–	–
82d	540	335	−205	38	142	120	−22	15	–	–	–	–
83b	319	321	+ 2	1	53	54	+ 1	2	–	–	–	–
84a	300	400	+100	25	–	–	–	–	–	–	–	–
84b	–	–		–	–	–	–		–	–	–	–
85	515	880	+365	41	–	–	–	–	–	–	–	–
86b	427	383	− 44	10	–	–	–	–	–	–		–
86c	510	437	− 73	14	–	–	–	–	–	–	–	–
87	235	441	+206	47	–	–	–	–	–		–	–
88	1003	668	−335	33	–	–	–	–	–		–	–
89	400	698	+298	43	–	–	–	–	–	–	–	–
4c	561	465	− 96	17	130	97	−33	25	–	–		–
90a	302	214	− 88	29	–	–			–	–	–	–
90b	–	–	–	–	–	–			–	–		–
91	–	–	–	–	–	–		–	–	–	–	–
92	683	375	−308	45	198	198	0	0	–	–	–	–
93	360	310	− 50	14	–	–	–	–	–	–	–	–
	GROUP B2 Renal disease (except pyelonephritis) with unilateral or mainly asymmetric involvement											
106	547	915	+368	40	–	–	–		–	–	–	–
107	534	457	− 77	14	–	–		–	–	–	–	–
108	433	4.8	− 5	1	–	–		–	–			–
109	–	–		–	–	–			–		–	
110	–	–		–	–	–	–		–	–	–	–
111	–	–		–	–	–	–	–	–		–	–
112	–	–		–	–	–		–	–		–	–
	GROUP B3 Arterial hypertension with stenosis or aneurysm of the main renal artery											
117a	478	404	− 74	15'	130	140	+10	7	63	41	−22	35
117b	357	365	+ 8	2'	56	61	+ 5	8	86	82	4	5
118	622	457	165	27	–	–		–	–			–
119	215	235	+ 20	9'	53	.8	25	47	16	.6	−10	38
1.0	614	564	− 50	8'	130	159	+29	18	100	61	39	39
121	262	234	− 28	11	93	116	+23	.0				
122	250	290	+ 40	14'	91	82	9	10	39	32	7	18
123	438	444	+ 6	1	68	71	+ 3	4	5	5	0	0
124	375	188	−187	50'	36	63	+27	43	31	8	23	74
125	287	261	− 26	9	80	82	+ 2	2	19	15	4	21
126	278	349	+ 71	20	88	86	− 2	2	23	.1	0	0
127	556	536	.0	4	176	160	+34	21	68	47	21	11
128	191	186	5	3	69	71	+ 2	3	10	9	1	10
129	–	–		–	–		–		–			
	GROUP B4 Renal disease with expected homogeneous involvement of both kidneys											
132	379	341	− 38	10	124	112	−12	10	27	.5	–	7
133	190	289	+ 99	34	34	40	+ 6	15	13	25	+12	48
134	–		–				–	–				
136	504	534	+ 30	6								
137	744	780	+ 36	5								
138a	475	460	− 15	3								
139	1030	1125	+ 95	8	–							
140	–	–			–					–		
141	–	–	–	–						–		
142	–	–		–						–		
143	–	–	–									
	GROUP B5 Patients with symptoms from the lower urinary tract in whom involvement of the kidneys could not be ruled out											
148	570	522	+ 2	0	–					–	–	
149	779	759	− .0	3	166	168	–	1	–	–	–	–
150	727	735	+ 8	1								

Table Xb (continued)

1	11				12		13		14		15		16			
Case no	Cin (ml min)				Urine culture (bact ml)		Roentgenol exam		Loin tenderness to palp		History of loin pain					
	R	L	Diff		R	L	R	L	R	L	R	L				
GROUP B1 Chronic pyelonephritis with signs of urinary tract obstruction (≥ 3 cardinal criteria)																
81	27	16	11	41	–	–	(+)	+	0	0	–	–				
82a	15	1	– 3	20	500 000	400 000	(+)	(+)	+	0	–	–				
82b	–		–	–	0	10 300	(+)	(+)	0	0	+	0				
82c	–	–	–	–	0	100 000	(+)	(+)	0	0	0	0				
82d	–			–	15 000	10 000	(+)	+	0	0	0	0				
83b	9	15	+ 6	40	–		+	(+)	0	(+)	–	–				
84a	7	15	+ 8	50	50 000	50 000	+	(+)	0	0	0	0				
84b	–			–	0	0	+	(+)	0	0	0	0				
85	–		–	–	0	0	+	0	0	0	+	0				
86b			–	–	4 700	3,500	(+)	+	0	0	0	0				
86c				–		–	–	–	0	0	0	0				
87	–		–		>100 000	>100 000	+	(+)	0	0	+	0				
88	–		–	–	0	500 000	(+)	+	0	+	0	+				
89		–		–		–	+	0	0	0	+	0				
4c			–		0	30 000	0	+	0	0	+	+				
90a	15	3	12	80	5 000	150 000	(+)	+	0	0	+	+				
90b		–		–	>100 000	>100 000	(+)	+	–	0	+	+				
91			–		0	–	0	+	0	0	0	+				
9			–		0	70 000	(+)	+	0	+	0	+				
93				–	0	30 000	0	(+)	0	0	0	0				
GROUP B2 Renal disease (except pyelonephritis) with unilateral or mainly asymmetric involvement																
106					0	0	+	0	+	0	+	0				
107	77	4	53	69	–	–	0	+	0	0	0	0				
108				–	0	0	0	0	0	0	–	–				
109							+	+	0	0	–					
110							(+)	+	0	0	+	+				
111	–				–		0	+	0	+	0	+				
112					–		0	+	0	0	0	0				
GROUP B3 Arterial hypertension with stenosis or aneurysm of the main renal artery													**Diuresis (ml min) R**	**L**	**Diff**	
117a	33	34	1	3	–	–	( )	(+)	+	(+)		–	0.64	1.76	–0.6[illegible]	49
117b	8	12	4	33	–			–				–	0.30	0.36	+0.06	17
118	1	66	45	68	–		+	0	0	0	–		0.06	1.10	+1.04	95
119			–			–	0		0	0	–		3.[illegible]5	0.87	–[illegible].38	73
120	37	44	+ 7	16		–	(+)	0	0	0		–	0.47	0.96	+0.49	51
121	79	6	3	10		–	0	–	0	0	–		–	–	–	–
122							+	0	0	0	–			–	–	–
123			–				+	0	0	0	–	–	0.64	0.43	0.[illegible]1	33
124							–	0	0	0		–	0.66	5.93	+5.[illegible]7	89
125							+	0	0	0		–	1.03	1.06	+0.03	3
126					–		+	+	0	0	–		2.05	0.73	–1.3	64
127	34	34	0	0			0	+	0	0	–		0.52	0.79	+0.27	34
128							+	0	0	0	–	–	3.07	1.53	–1.54	50
129					–		+	0	0	0		–	–	–	–	–
GROUP B4 Renal disease with expected homogeneous involvement of both kidneys																
130				–	0	0	0	0	0	0			2.11	2.16	+0.05	–
131					–		0	0	0	0			0.95	0.9	–0.03	3
134							0	0	0	0	0	0				
136						–	0	0	0	0	–	–				
137	49	63	14	–			0	0	0	0	–	–				
138	5	16	9	36				–	0	0	0	0				
139					0	0	0	0	0	0	–	–				
140					–	–	0	0	0	0	0	0				
141						–	0	0	0	0	0	0				
142							0	0	0	0	0	0				
143					–	–	0	0	0	0						
GROUP B5 Patients with symptoms from the lower urinary tract in whom involvement of the kidneys could not be ruled out																
148	–				–	–	0	0	0	0	0	0				
149		–			–	–	0	0	0	0	–	–				
150		–	–	–	0	0	0	0	(+)	+	0	0				

* Am chlor load

ED % R	L	Diff	
74.7	74.6	–0.1	0

Table Xb (continued)

1	8				9		10			
Case no	RPF (ml/min)				CPAH (ml/min)		Ccr (ml/min)			
	R	L	Diff	%	R	L	R	L	Diff	
	GROUP B1 Chronic pyelonephritis with signs of urinary tract obstruction (≥ 3 cardinal criteria)									
81	162	111	− 51	31	121	77	34	22	−12	35
82a	–	–	–	–	70	79	14	15	+ 1	7
82b	–	–	–	–	–	–	–	–	–	–
82c	–	–	–	–	–	–	–	–	–	–
82d	–	–	–	–	–	–	–	–	–	–
83b	206	101	−105	51	23	37	9	14	+ 5	36
84a	34	66	+32	48	22	50	8	19	+11	58
84b	–	–	–	–	–	–	–	–	–	–
85	–	–	–	–	–	–	–	–	–	–
86b	–	–	–	–	–		–			–
86c	–	–	–	–	–		–	–	–	–
87	–	–	–	–	15	57	11	34	+23	68
88	–	–	–	–	–	–	–	–	–	–
89	–	–	–		–	–	–	–		–
4c	–	–	–		–			–		–
90a	143	76	− 67	47	47	8	15	3	−12	80
90b	–	–	–		–	–	–	–		–
91	–	–	–		–	–	–	–		–
92	–	–	–		–	–	–	–	–	–
93	–	–	–		–	–				–
	GROUP B2 Renal disease (except pyelonephritis) with unilateral or mainly asymmetric involvement									
106		–	–	–	–	–	–			
107		–	–	–	238	15	53	18	−35	66
108		–	–	–	–			–	–	–
109	–	–	–	–	–	–		–		–
110	–	–	–		–	–	–			–
111	–	–	–		–	–	–	–	–	
112	–	–	–		–			–		
	GROUP B3 Arterial hypertension with stenosis or aneurysm of the main renal artery									
117a	203	108	− 95	47	146	153	26	24	− 2	8
117b	–	–	–	–	166	193	10	13	+ 3	3
118	26	193	+167	87	31	159	9	33	+ 4	73
119	–	–			–	–	35	17	18	50
120	293	418	+125	30	245	341	29	41	−12	29
121	–		–		143	151	31	35	− 4	11
122		–		–	–	–	–			
123	273	184	− 89	33	239	[illegible]	55	40	15	27
124			–	–	–		30	50	0	40
125		–	–		–	–	70	49	21	30
126		–	–		–		26	46	[illegible]	43
127	181	167	14	8	146	141	34	27	7	21
128	–	–	–		[illegible]	61	14	30	16	53
129	–		–			–				–
	GROUP B4 Renal disease with expected homogeneous involvement of both kidneys									
132	–	–		–	–		63	50	13	21
133	–	–	–		169	234	51	61	+ 10	16
134	–	–	–		–					
136	–	–		–						
136	379	442	+ 63	14	315	387	45	[illegible]	17	7
137	–		–		170	126	23	17	6	−6
138a	–	–	–		–	–				
139	–	–	–		–	–				
140	–									
141	–	–	–	–	–	–		–		
142	–	–	–	–	–	–		–		
143	–	–						–		
	GROUP B5 Patients with symptoms from the lower urinary tract in whom involvement of the kidneys could not be ruled out									
148	–	–	–	–	–	–	–	–		–
149	–	–	–	–	–		–	–		
150	–	–	–	–						

Table Xb (*continued*)

1	11				12		13		14		15		16			
Case no	C_{In} (ml min)				Urine culture (bact/ml)		Roentgenol exam		Loin tenderness to palp		History of loin pain					
	R	L	Diff		R	L	R	L	R	L	R	L				
GROUP B1 Chronic pyelonephritis with signs of urinary tract obstruction (≥ 3 cardinal criteria)																
81	77	16	−11	41		—	(+)	+	0	0	–	–				
82a	15	12	− 3	20	500 000	400 000	(+)	(+)	+	0	–	–				
8 b	–	–	–	–	0	10 300	(+)	(+)	0	0	+	0				
8.c	–			–	0	100 000	(+)	(+)	0	0	0	0				
8.d	–	–		–	15 000	10 000	(+)	+	0	0	0	0				
83b	9	15	+ 6	40			+	(+)	0	(+)	–	–				
84a	7	15	+ 8	50	50 000	50 000	+	(+)	0	0	0	0				
84b		–	–	–	0	0	+	(+)	0	0	0	0				
85	–			–	0	0	+	0	0	0	+	0				
86b	–	–	–		4 700	3 500	(+)	+	0	0	0	0				
86c				–			–		0	0	0	0				
87	–		–		>100 000	>100 000	+	(+)	0	0	+	0				
88	–	–	–	–	0	00 000	(+)	+	0	+	0	+				
89				–		–	+	0	0	0	+	0				
4c		–	–	–	0	30 000	0	+	0	0	+	+				
90a	15	3	12	80	5 000	150 000	(+)	+	0	0	+	+				
90b					>100 000	>100 000	(–)	+	+	0	+	+				
91			–	–	0		0	+	0	0	0	+				
9				–	0	70 000	(+)	+	0	+	0	+				
93					0	30 000	0	(+)	0	0	0	0				
GROUP B2 Renal disease (except pyelonephritis) with unilateral or mainly asymmetric involvement																
106					0	0	+	0	+	0	+	0				
107	77	4	53	69			0		0	0	0	0				
108				–	0	0	0	0	0	0						
109				–	–		+	+	0	0	–	–				
110							( )		0	0	+					
111							0		0		0	–				
11							0	+	0	0	0	0				
GROUP B3 Arterial hypertension with stenosis or aneurysm of the main renal artery													Diuresis (ml min) R	L	Diff	
117a	33	34	1	3			( )	( )		(+)	–		0.64	1.26	+0.6	49
117b	8	1	4	33			–			–		–	0.30	0.36	+0.06	17
118	1	66	45	68			+	0	0	0		–	0.06	1.10	−1.04	95
119							0		0	0		–	3.–5	0.87	–.38	73
120	37	44	7	16			( )	0	0	0	–		0.47	0.96	+0.49	51
1 1	79	6	3	10			0		0	0		–		–	–	–
1								0	0	0			–	–	–	–
1 3								0	0	0	–	–	0.64	0.43	−0.71	33
1 4								0	0	0	–		0.66	5.93	+5. 7	89
1 5								0	0	0		–	1.03	1.06	+0.03	3
1 6								–	0	0			.05	0.73	−1.3	64
1 7	34	34	0	0			0	+	0	0	–		0.5	0.79	0. 7	34
1 8								0	0	0	–	–	3.07	1.53	−1.54	50
1?9								0	0	0	–	–	–		–	–
GROUP B4 Renal disease with expected homogeneous involvement of both kidneys																
13					0	0	0	0	0	0						
133							0	0	0	0	–		2.11	2.16	0.05	–
134							0	0	0	0	0	0	0.95	0.9	0.03	3
136							0	0	0	0	–					
137	49	63	14				0	0	0	0	–					
138a	25	16	9	36					0	0	0	0				
139					0	0	0	0	0	0	–	–				
140							0	0	0	0	0	0				
141							0	0	0	0	0	0				
14						–	0	0	0	0	0	0				
143					–	–	0	0	0	0		–				
GROUP B5 Patients with symptoms from the lower urinary tract in whom involvement of the kidneys could not be ruled out																
148	–				–	–	0	0	0	0	0	0				
149							0	0	0	0		–				
1.0					0	0	0	0	(+)	+	0	0				

Am chlor load

E_D *			
R	L	Diff	
74.7	74.6	−0.1	0

Table Xb *(continued)*

1	2				3				4			
Case no	Max conc ability (mOsmol/lit)				UNa (mEq/lit)				UK (mEq/lit)			
	R	L	Diff		R	L	Diff	/	R	L	Diff	
GROUP B1 Chronic pyelonephritis with signs of urinary tract obstruction (≥ 3 cardinal criteria)												
94	370	368	– 2	1	75	82	+ 7	9	–	–	–	–
95	493	461	– 32	6	–	–	–	–	–	–	–	–
96	635	933	+298	32	–	–	–	–	–	–	–	–
97	413	853	+440	52	91	177	+ 86	49	–	–	–	–
98	568	1075	+507	47	–	–	–	–	–	–	–	–
99a	525	480	– 45	9	–	–	–	–	–	–	–	–
99b	380	475	+ 95	20	84	83	– 1	1	–		–	–
100	750	1048	+298	28	–	–	–	–	–	–	–	–
101	245	1000	+755	76	110	230	+120	52	–	–	–	–
102a	441	436	– 5	1	–	–	–	–		–	–	–
102b	458	448	– 10	2	125	123	– 2	2	–			–
103	545	492	– 53	10	170	164	– 6	4	–		–	–
104	500	473	– 27	5	132	134	+ 2	1	–		–	–
105a		–	–	–	21	19	– 2	10	16	28	+12	43
105b	384	331	– 53	14	–	–	–	–	–	–	–	–
GROUP B2 Renal disease (except pyelonephritis) with unilateral or mainly asymmetric involvement												
113	796	488	–308	39	134	117	– 17	13				–
114	613	643	+ 30	5	170	180	+ 10	6		–		–
115	370	342	– 28	8	102	102	0	0	–		–	–
116b	820	450	–370	45	–	–						
GROUP B3 Arterial hypertension with stenosis or aneurysm of the main renal artery												
130	228	220	– 8	4	4	5	+ 1	20	2	2	0	0
131	–	–		–	24	25	+ 1	4				–
GROUP B4 Renal disease with expected homogeneous involvement of both kidneys												
135	515	520	+ 5	1	187	183	4	2	–			–
144	528	520	– 8	2	–	–	–			–		–
145	298	296	– 2	1	90	100	+ 10	10				–
146	548	488	– 60	12	105	108	+ 3	3	–	–		
147	390	435	+ 45	10	74	69	– 5	7				
GROUP B5 Patients with symptoms from the lower urinary tract in whom involvement of the kidneys could not be ruled out												
151	855	945	90	10	240	230	10	4	60	62	2	3
152	928	955	27	3	192	183	9	5				

Table Xb *(cont nued)*

1	5				6			7		8		9		10	
Case no	Uc (mEq/l t)				UpH			Ur ne culture (bact/ml)		Roentge nol exam		Lo n tender ness to palp		H story of loin pain	
	R	L	D ff		R	L	Diff	R	L	R	L	R	L	R	L
GROUP B1 Chron c pyelonephr tis with s gns of ur nary tract obstru ction (≥ 3 card nal cr teria)															
94								3 2 m 1	780 000	+	+	0	+	(+)	+
95								0	0	+	(+)	(+)	0	+	0
96								0	0	0	0	+	0	+	(+)
97								35 000	0	+	0	0	0		
98								?00 000	0	+	0	0	0	+	0
99a								0	160 000	(+)	+	0	0		
99b	70	85	+15	18				100 000	900 000	(+)	+	0	0	0	0
100								0	300	+	0		0	+	0
101								>100 000	0	+	0	+	0	+	0
0.a								0	0	+	+	0	0		
0 b								0	0	+	(+)	0	0	0	0
103	70	60	10	14				0	0	0	(+)	0	+	0	+
104	75	65	10	13				0	0	(+)	+	+	+	+	
05a	10	35	+25	71				0	0	+	(+)	0	+	0	0
0 b								9 000	0 000	+	(+)	0	0	0	0
GROUP B2 Renal d sease (except pyelonephr t s) w th un lateral or n a nly asym n etr c invol en ent															
113								0	80 000	0	+	0	0	0	0
1 4								5 000	0	+	0	+	0	+	0
115								0	70 000	+	+	0	+	0	0
6b										0	0	0	+	+	+
GROUP B3 Arter al hypertens on w th stenos s or aneurysm of tl e n a n renal artery															
30	15	15	0	0						+	(+)	0	0		
131	15	15	0	0						+	0	0	0		
GROUP B4 Renal d sease w th expected l on ogeneo s n ol en ent of both k dneys															
35								0	?00	0	0	0	0	0	0
144								0	0	(+)	(+)	0	0	0	0
45					7 6	6 8	0 8	0	0	(+)	(+)	0	0	(+)	0
46	80	75	5	6				0	0	0	0	0	0	0	0
147								0	0	+	+	0	0	0	0
GROUP B5 Pat ents w th sympton s from the lower ur nary tract in wl om involvement of the k dneys could not be ruled o t															
51										0	0	0	0	0	0
15								0	0	0	0	0	0	0	0

Table XIa *Symmetry and asymmetry of individual renal functions and of other data*

1	2	3	4	5	6	7	8	9	10	11	12	13
Case no	U_{Osmol} >10 %	U_{Na} ≥10 %	U_K ≥10 %	U_{Cr} >20 %	U_{pH} ≥0 05	E_{PAH} ≥3 %	RPF ≥20 %	C_{Cr} ≥20 %	C_{In} >20 %	Urine culture ≥10×	Roentgenol exam	Loin tenderness to palp
GROUP A1 Chronic non obstructive pyelonephritis (≥ 3 cardinal criteria)												
1	=	↓	–	–	–	↓	–	–	–	↑		[=]
2a	=	–	–	–	↑	↓	–	–	–		=	[=]
2b	=	–	–	–	–	–	–	–	–		↑	[=]
3	↓	↓	–	↓	–	↑	–	–	–	↓	↓	[=]
4a	↓	–	–	–	=	↓	=	=	↓		=	[=]
4b	=	–	–	–	–	=	–	–	–	↓	–	[=]
5	↓	–	–	–	–	–	–	–	–	↑	↓	[=]
6b	↓	–	–	–	–	–	–	–	–	↓	↓	=
6c	=	↑	–	↓	–	↓	–	–	–	[–]	↓	[=]
7a	↑	–	–	–	–	↓	–	–	–	[–]	[=]	[=]
7b	=	=	–	=	–	–	–	–	–		[=]	[=]
8a	–	–	–		=	↓	=	↓	=	–	[=]	[=]
8b	–	–	–	–	–	↓	–	–	–	–	↓	[=]
9a	=	–	–	–	↓	↓	–	↓	↓	–	↓	[=]
9b	=	–	–	=	–	↓	–	–	–	[–]	↓	[=]
10	↑	–	–	–	=	↓	–	–	–		[=]	[=]
11	–	–	–	–	–	↓	–	=	↑	–	[–]	=
12	↑	–	–	–	↑	↑	=	↑	↑	–	↑	[=]
13a	↓	–	–	–	=	=	=	↓	=	–	↓	[=]
13b	=	=	–	–	–	[=]	–	–	–	[–]	↓	[=]
14a	=	–	–	–	–	↓	–	–	–	–	↓	[=]
14b	=	–	–	–	–	–	–	–	–		↓	[=]
14c	↑	↓	–	–	–	↓	–		–	[–]	=	[=]
15	↓	–	–	–	–	↓	–	–	–	–	↓	↓
16	↑	=	–	↑	–	↑	–	–	–	↑	↑	[=]
17	↑	–	–	–	–	↓	–	–	–	↓	↓	[=]
18	=	–	–	–	–	↓	–	–	–	↑	↑	=
19a	↑	–	–	–	–	=	↓	↓	↓		↑	[=]
19b	↑	↑	–	–	–	=	–	–	–	–	↑	[=]
20a	↓	–	–	–	–	↓	=	↓	↓	–	↓	↓
20b	↓	–	–	–	–	–	–	–	–		↓	↓
21	↓	=	↓	–	–	–	–	↑	–	↓	↓	↑
22a	↓	–	–	–	–	↓	↓	↓	↓		↓	[=]
22b	–	↑	–	↓	–	↓	–	–	–	–	↓	↑
23	↑	↑	–	↑	–	–	–		–	↑	↑	[=]
24	↑	–	–	–	–	–	–	–	–	↓	↓	↑
25a	↑	–	–	–	–	–	–	–	–	↑	[–]	[=]
25b	↑	=	–	↑		–	–		–	=	↑	[=]
26b	↑	–	–	–	–	–	–	–	–		↑	↓
27	=	–	–	–		↓	–		–	[–]	↓	[–]
28c	–	–	–		–	↓		–	–	–		↑
29c		–	–	–		↓	–			–	↑	[=]
30b	–	–	–	–	–	↓	–	–	–	–	–	↑
31	–	–	–	–		[=]	–		–	–	[ ]	
GROUP A2 Acute and suspected chronic non-obstructive pyelonephritis (< 3 cardinal criteria)												
70	↑	–	–	–		↑	–			[–]	[ ]	[–]
71		–	–	–	↓	↓	–	=	[=]	–	[ ]	[=]
72	[–]	=	–	–		[ ]	–		–	[ ]	[=]	[ ]

Symbols
= Symmetry with reduced function
[=] Symmetry with function within normal limits
↑ *Asymmetry* with poorer function of right kidney
↓ Asymmetry with poorer function of left kidney
– Not tested

The borderline of symmetry of the relevant function is given in each heading

Column 1
a, b c and d denote examinations on different occasions in the same patient

Table XIa *(continued)*

1	2	3	4	5	6	7	8	9	10
Case no	U_{Osmol} ≥10 ,	U_{Na} –10%	U_K ≥10%	U_{Cr} ≥20	U_{pH} ~0 05	Urine culture	Roentgenol exam	Loin tenderness to palp	History of loin pain
GROUP A1 Chronic non-obstructive pyelonephritis (≥ 3 cardinal criteria)									
32	=	=	–	=	–	=	=	↓	↓
33	↑	↑	–	–	–	[=]	[=]	[=]	–
34	=	=	–	–	–	↓	↓	=	↓
35	=	=		=	–	[=]	–	[=]	–
36	=		–	–	–	–	[=]	[=]	↑
37	=	=	–	=	–	[=]	[=]	[=]	–
38	↓	↓	–	↓	–	↓	↓	↓	↓
39	↓			–	–	[=]	↑	[=]	[=]
40	↓	–	–	–	–	↑	↑	↑	
41	↑		–	–	–	=	=	[=]	[=]
42	↑	=		=	↑	–	[=]	[=]	–
43	↑	↑	–	–	–	[=]	[=]	↑	↑
44	↑			=	↑	[=]	↑	[=]	–
45	↑			–	–	–	↑	[=]	↑
46	↓	–	–		–	[=]	=	↓	=
47	=			–	–	=	[=]	[=]	↓
48	=	=	–	=	↑	[=]	[=]	[=]	↓
49	=	=	–		↑	[=]	[=]	↑	↑
50	↓	↓		↓	–	–	=	[=]	–
51	↓		–	↓	↓	↓	↓	[=]	↓
5	↓	=		=	↓	[=]	↓	[=]	–
53	↑	↑		=	↑	[=]	[=]	=	↑
54	↑		–	–	–	=	↓	[=]	–
55	=	↑	–	↑	–	[=]	↓	=	↑
56	↓	=		=	=	=	=	=	↓
57	=	=		=	–	↓	[=]	=	–
58	↑	↑	–	=	↓	↑		[=]	–
59	=			–	–	↑	=		↑
60	↓	=	↓	–	–	[=]	↓	↑	↑
61	=		–			↓	↑	↑	↑
6	↓	–	–	–	–	↓	↓	↓	↓
63	↑	–	–	–	–	=	=	[=]	–
64		–		–	–	[=]	↓	[=]	[=]
65	↓	–			–	↓	=	↑	↑
66	=	–			–	=	↓	=	–
67	=	↓		=	–	[=]	[=]	↓	=
68	[=]	–			–	[=]	[=]	[=]	↑
69	=	↑		=	–	–	[=]	[=]	[=]
GROUP A2 Acute and suspected chronic non-obstructive pyelonephritis (< 3 cardinal criteria)									
73	[=]	=	–		–	↓	[=]	[=]	[=]
74	[=]		–		–	[=]	[=]	[=]	–
75	↓	↓			–	[=]	[=]	[=]	[=]
76		↓			–	[=]	[=]	[=]	↓
77	↓	=			–	[=]	↓	[=]	[=]
78	=	↑		↓	–	[=]	↓	[=]	[=]
79	↑	=		=	–	[=]		[=]	[=]
80	[=]	=		=	–	[=]	[=]	[=]	–

Table XIb *Symmetry and asymmetry of individual renal functions and of other data*
Symbols as in Table XIa

1	2	3	4	5	6	7	8	9	10	11	12	13	14	1[illegible]
Case no	U_{Osmol} ≥10 %	U_{Na} ≥10 %	U_K ≥10 %	U_{Cr} ≥20 %	U_{pH} ≥0 05	E_{PAH} ≥3 %	RPF >20 %	C_{Cr} ≥20 %	C_{In} ≥20 %	Urine culture ≥10×	Roentgenol exam	Loin tenderness to palp	History of loin pain	Diur ml [illegible] >%
GROUP B1 Chronic pyelonephritis with signs of urinary tract obstruction (≥ 3 cardinal criteria)														
81	=	–	–	–	–	↓	↓	↓	↓	–	↓	[=]	–	
82a	–	–	–	–	–	–	–	=	↓	=		↑	–	
82b	=	–	–	–	–	–	–	–	–	↓	=	[=]	↑	
82c	↓	↓		=	–	–	–	–	–	↓	–	[=]	[=]	
82d	↓	=	–	↑	↓	–	–	–	–	~	↓	[=]	[=]	
83b	–	–	–	–	↓	↑	↓	↑	↑	~	↑	↓	–	
84a	↑	–	–	–	–	↑	↑	↑	↑	~	↑	[=]	[=]	
84b	–	–	–	–	–	=	–	–	–	[~]	↑	[ ]	[=]	
85	↑	–	–	–	–	↑	–	–	–	[~]		[–]		
86b	↓	–	–	–	–	–	–	–	–	≈	↓	[–]	[=]	
86c	↓	–	–	–	–	↓	–	–	–	~	–	[–]	[~]	
87	↑	–	–	–	–	↑	–	–	–	≈	↑	[=]	↑	
88	↓	–	–	–	–	[=]	–	–	–	↓	↓	↓	↓	
89	↑	–	–	–	–	–	–	–	–	~	↑	[=]	↑	
4c	↓	↓	–	=	–	↓	–	–	–	↓	↓	[=]	=	
90a	↓	–	–	–	–	↓	↓	↓	↓	↓	↓	[–]	=	
90b	–	–	–	–	–	↓	–		–	=	↓	↑	–	
91	–	–	–	–	–	↓	–		–		↓	[–]	↓	
92	↓	≈	–	↓	↓		–		–	↑	↑	↑	↑	
93	↓	–	–	–	–		–		–	↓		[–]	[=]	
GROUP B2 Renal disease (except pyelonephritis) with unilateral or mainly asymmetric involvement														
106	↑	–	–			↓	–		–	[~]	↑	↑	↑	
107	↓	–	–		↓	↓	–	↓	↓		↓	[=]	[~]	
108	–	–	–			↑	–	–	–	[~]	[–]	[–]	–	
109	–	–	–	–		↓	–	–	–	–	–	[=]		
110	–	–	–	–	–	↑			–	–	↓	[=]		
111	–	–	–	–	–	↓				–	↓	↓	↓	
112	–	–	–	↑	–	↓	–	–	–		↓	[–]	[ ]	
GROUP B3 Arterial hypertension with stenosis or aneurysm of the main renal artery														
117a	↓	=	↓		–	↓	↓					↑	–	=
117b	=	=	=	↑				↑	↑					=
118	↓		–			↑	↑	↑	↑		↑	[ ]	–	↑
119	=	↓	↑		–	↓		↓			↓	[ ]		↓
120	=	↑	↓	–	–	=	↑	↑	=		↑	[ ]	–	
121	↓	↑		–	–	↓		–	=		↓	[–]		
122	↑	↓	↓	↓		[=]		–			↑	[–]		=
123		=	=			↓	↓	↓	–		↑	[–]		
124	↓	↑	↓	–		[=]		↑			↑	[–]		=
125	=	=	↓			↓		↓	–		↑	[ ]		
126	↑	=	–			↓		↑	–			[=]	–	↓
127	=		↓	–		↑					↓	[ ]		=
128	=		↓					↑			↑	[=]		
129				–		↓	–				↑	[–]		
GROUP B4 Renal disease with expected homogeneous involvement of both kidneys														
132	↓	↓		–		↓		↓	–	[=]	[ ]	[ ]		=
133	↑	↑	↑	–		↓					[=]	[ ]	[=]	
134	–	–	–			↓					[ ]	[ ]		
136		–		–	=	↑	=	↑	↑	–	[ ]	[ ]		
137	=				↓	↑		↓	↓		[=]	[ ]	[=]	
138a	=	–	–			[ ]	–			[=]	[=]	[=]		
139	[–]	–	–		–	[ ]				–	[=]	[=]	[=]	
140	–	–	–				–			–	[=]	[=]	[=]	
141	–	–	–	–		↓				–	[=]	[=]	[=]	
142	–	–	–			↓				–	[=]	[=]		
143	–	–	–											
GROUP B5 Patients with symptoms from the lower urinary tract in whom involvement of the kidneys could not be ruled out														
148	=	–	–	–	↑	↑				–	[=]	[=]	[=]	
149	=	–	–	–		[ ]				[=]	[=]	[=]	[=]	
150	=	=	–											

Table XIb (*continued*)

1	2	3	4	5	6	7	8	9	10
Case no	U_{Osmol} ≥10	U_{Na} ≥10	U_{Y} ≥10	U_{Cr} ≥20	U_{pH} >0 05	Urine culture ≥10×	Roentgenol exam.	Loin tenderness to palp	History of loin pain
GROUP B1 Chronic pyelonephritis with signs of urinary tract obstruction (≥ 3 cardinal criteria)									
94	−	=	−	−	−	=	≈	↓	↓
95	=	−	−	−	−	[ ]	↑	↓	↓
96	↑	−	−	−	−	[=]	[≈]	↓	↓
97	↑	↑		−		↑	↑	[=]	−
98	↑	−	−	−	−	↑	↑	[=]	
99a	=	−		−	−	↓	↓	[=]	−
99b	↑	=		=	−	=		[=]	[=]
100	↑	−	−		−	[=]	↑	↑	↑
101	↑	↑		−	−	↑	↑	↑	↑
10.a		−	−	−		[−]	=	[=]	−
10 b	=	=	−	−	−	[ ]	↑	[=]	[=]
103	↓	=		=	−	[=]	↓	↓	↓
104	=	=	−	=	−	[=]	↓	=	=
105a	−	↓	↑	↑	−	[ ]	↑	↓	[=]
105b	↓	−		−		↓	↑	[=]	[=]
GROUP B2 Renal disease (except pyelonephritis) with unilateral or mainly asymmetric involvement									
113	↓	↓		−	−	↓	↓	[=]	[=]
114	=	=	−	−		↑	↑	↑	↑
115	=	=		−	−	↓	=	↓	=
116b					−	−	[=]	↓	=
GROUP B3 Arterial hypertension with stenosis or aneurysm of the main renal artery									
130	=	↑	=	=			↑	=	−
131	−	≈	−	=	−	−	↑	=	
GROUP B4 Renal disease with expected homogeneous involvement of both kidneys									
135	=	≈			−	[ ]	[=]	[=]	[ ]
144	=			−	−	[=]	=	[=]	[ ]
145	=			−	↑	[ ]	=	[=]	↑
146		≈	−	=		[=]	[=]	[=]	[ ]
147		≈	−		−	[ ]	=	[=]	[=]
GROUP B5 Patients with symptoms from the lower urinary tract in whom involvement of the kidneys could not be ruled out									
151	↑	=	=		−		[=]	[=]	[=]
15	[=]	=		−	−	[ ]	[=]	[=]	[=]

Table XII *Incidence of symmetry and asymmetry of the individual renal functions and of other data in the different groups of diseases*

When evaluating the GFR, only the C_{Cr} *was taken into account and the* C_{In} *was omitted (cf p 69)*

	U_{Osmol}							U_{Na}						
	A1	A2	B1	B2	B3	B4	B5	A1	A2	B1	B2	B3	B4	B5
Normal symmetry	1	4	—	—	—	1	1	—	—	—	—	—	—	—
Pathol symmetry	30	2	10	3	8	6	3	19	5	7	2	7	3	3
Consistent asymmetry	33	4	20	4	2	2	—	13	2	4	1	5	3	—
Inconsistent asymmetry	2	1	—	—	3	—	—	2	1	1	—	2	—	—
Indecisive	10	—	1	—	1	2	1	2	—	—	—	—	—	—
No of invest	76	11	31	7	14	11	5	36	8	12	3	14	6	3

	U_{Cr}							E_{PAH}						
	A1	A2	B1	B2	B3	B4	B5	A1	A2	B1	B2	B3	B4	B5
Normal symmetry	—	—	—	—	—	—	—	2	1	1	—	2	2	1
Pathol symmetry	15	2	5	—	2	1	—	4	—	1	—	2	2	—
Consistent asymmetry	10	2	2	—	2	—	—	16	2	9	3	6	2	1
Inconsistent asymmetry	—	—	1	1	—	—	—	3	—	—	1	1	2	—
Indecisive	—	—	—	—	—	—	—	7	—	1	3	2	3	1
No of invest	25	4	8	1	4	1	0	32	3	12	7	13	11	3

	RPF							C_{Cr}						
	A1	A2	B1	B2	B3	B4	B5	A1	A2	B1	B2	B3	B4	B5
Normal symmetry	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Pathol symmetry	5	—	—	—	1	1	—	2	1	1	—	2	1	—
Consistent asymmetry	1	—	3	—	4	—	—	6	—	4	1	9	3	—
Inconsistent asymmetry	—	—	—	—	—	—	—	1	—	—	—	—	—	—
Indecisive	1	—	1	—	—	—	—	1	—	1	—	1	—	—
No of invest	7	0	4	0	5	1	0	10	1	6	1	12	4	0

	Urine culture							Roentgenol exam						
	A1	A2	B1	B2	B3	B4	B5	A1	A2	B1	B2	B3	B4	B5
Normal symmetry	22	9	10	2	—	7	2	20	8	1	2	—	12	5
Pathol symmetry	16	—	8	—	—	—	—	14	—	5	2	2	3	—
Consistent asymmetry	15		11	3	—		—	38	3	22	6	8	—	—
Inconsistent asymmetry	3	—	—	—	—	—	—	1	—	1	—	3	—	—
Indecisive	4	1	1	—	—	—	—	8	—	5	1	2	—	—
No of invest	60	10	30	5	0	7	2	81	11	34	11	15	15	5

	Loin tenderness to palp							History of loin pain						
	A1	A2	B1	B2	B3	B4	B5	A1	A2	B1	B2	B3	B4	B5
Normal symmetry	51	11	22	6	12	16	4	26	5	11	3	—	9	4
Pathol symmetry	9	—	1	—	2	—	—	8	—	4	3	—	—	—
Consistent asymmetry	15	—	8	5	—	—	—	19	2	13	3	—	1	—
Inconsistent asymmetry	4	—	2	—	1	—	—	4	—	—	—	—	—	—
Indecisive	3	—	2	—	—		1	7	1	1	—	—	—	—
No of invest	82	11	35	11	15	16	5	64	8	29	9	0	10	4

CASE 17 The difference between the concentration ability of the kidneys was 10 per cent, the right kidney being poorer in this respect On the pyelograms the outline of the left kidney was diffuse the density of contrast medium was greatly reduced bilaterally but more on the left side Table X shows that results of urine culture and of PAH extraction gave the same indications as the results of roentgenologic examination The history indicated on the contrary that the right kidney had been involved more often and more severely

In the aforegoing case serving as an example there were signs that both kidneys were greatly damaged by chronic pyelonephritis and the differences in the various tests were generally small This can explain why certain investigations gave contradictory information about which kidney was more involved The results of determinations of the osmolarity of the urine differed from those of other bilateral tests in one more patient in group A1 (case 40) and one patient in group A2 (case 76)

Determination of the renal PAH extraction showed divergent asymmetry in the following patients

CASE 3 The left kidney showed poorer concentration ability as well as a lower urinary concentration of Na and creatinine Moreover this kidney was smaller on the pyelograms (length of right kidney 14 cm, of left 1? cm) and had more pronounced anatomic changes than the right The density of contrast medium was however about the same bilaterally The PAH extraction by the right kidney was lower than that by the left whereas the Diodrast extraction was inappreciably higher on the right side It was evident from the history that there had been greater involvement of the right kidney

CASE ?9 The renal extraction of PAH was at variance with the roentgenologic features history and findings at palpation Thus the extraction by the left kidney was poorer whereas roentgenologic examination showed the right kidney to be smaller (length of right kidney 10 5 cm, of left 12 cm) and to have a thinner parenchyma The history also gave the same indications with localization of tension and pain to the right loin, which was also tender to palpation

CASE 138 The PAH extraction by the right kidney was greatly reduced (to 18 8 per cent) whereas that by the left was only slightly reduced (to 77 3 per cent) Two extraction determinations could be made on the right side but the lower value deviated from the mean by 27 per cent. This uncertain value is marked by an asterisk in Table X (*cf* Chap VIII) However the clearance of endogenous creatinine and of inulin showed poorer function of the left kidney This was also borne out by determination of the urinary pH although the difference was extremely small (0 05) Neither determination of the osmolarity of the urine the history nor palpation over the loins provided evidence of any difference between the kidneys The patient died just over a year after the investigation and autopsy also failed to establish any greater damage to either kidney Thus both were of the same size and each weighed 100 g Histologic examination showed signs of subchronic glomerulonephritis — In this case the suspicion arises that some technical or other error might have been responsible for the great discrepancy in PAH extraction by the right kidney

In 4 more cases (18 106 127 and 133) the extraction rate of PAH differed from those of other bilateral tests

The results are summarized in Table XII and are discussed in Chapter X (pp 110—117)

CHAPTER X

GENERAL DISCUSSION

Evaluation of the present results particularly with respect to the existence of any asymmetry of renal function was based chiefly on the PAH extraction and the renal concentration ability The reasons for this choice were

1 The tests are relatively easy to perform

2 The test of concentration ability does not require quantitative collection of the urine (164)

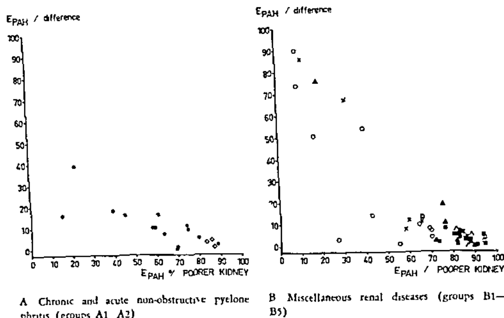
3 The tests provide information about the function of different parts of the nephron This is because extraction (59) is dealt with mainly by the proximal convoluted tubules (cortex), and concentration by the loop of Henle and the collecting tubules (medulla), through the counter current mechanism

Naturally, it would have been desirable to include the inulin and/or endogenous creatinine clearance as a measure of the glomerular filtration rate and the renal plasma flow as a measure of the renal blood flow These determinations could however be made only on a limited scale and the same degree of reliability cannot be ascribed to the results as to those of the renal extraction and concentration tests

A prerequisite for a clearance determination to be reliable is that the ureteric catheters are in position for a sufficiently long time to permit at least two (preferably three) clearance periods to be determined In most of my cases, the renal concentration ability was determined on the same occasion as the clearances so that the patient did not have to be catheterized twice at a short interval The patient was therefore allowed no fluids for 24 hours before the test and was also given an injection of vasopressin (Pitressin®) on the previous evening Consequently the urinary output was extremely small This implies both that the clearance periods would have had to be comparatively long to decrease the error due to dead space and that even small losses of urine caused by leakage beside the catheter would have been a greater source of error than with ample diuresis

For these reasons the glomerular filtration rate and renal plasma flow could not be estimated in some cases In those cases in which the determinations were made the results cannot be regarded to be as reliable as determinations of the renal concentration ability and PAH extraction

The other tests that were made were also judged to be of less consequence than the extraction and concentration abilities since they require further standardization of the conditions to be accorded full value For example the pH of the urine is of limited value since no ammonium chloride load was used so that the ability to excrete hydrogen ions was not maximally utilized Analogously the excretion of ammonia is difficult to assess since the glutaminase in the kidney and possibly other ammonia forming enzymes as well were not completely utilized



A Chronic and acute non-obstructive pyelonephritis (groups A1 A2)

B Miscellaneous renal diseases (groups B1—B5)

Fig 2 Difference (r_c) between the PAH extraction by the two kidneys (ordinate) and the extraction ratio (r_c) of the poorer kidney (abscissa) — For symbols see p 39

A Implications of Asymmetry of Individual Functions

In the majority of patients with chronic pyelonephritis there was measurable asymmetry of renal function An account of the criteria of asymmetry applied in the present series is given in Chapter I It seemed appropriate to investigate the extent to which the degree of asymmetry was related to the degree of renal damage It could be expected, *a priori*, to be more pronounced in chronic pyelonephritis with an increasing degree of renal damage since it seemed unlikely that the damage would involve both kidneys to the same extent

Asymmetry of PAH extraction

The aforementioned presumption did in fact apply However it seemed by no means to be specific to pyelonephritis Figure 2A shows the degree of asymmetry of PAH extraction in the cases of chronic and acute non-obstructive pyelonephritis (groups A1 A2) in relation to the extraction by the kidney that was poorer in this respect The degree of asymmetry is given as the difference in extraction value between the kidneys expressed as a percentage of the extraction by the better one This was considered to give a more correct idea of the asymmetry than the absolute difference It is evident that if the extraction by one kidney was 30 per cent and that by the other 60 per cent the asymmetry is more marked than if the values were 65 and 95 per cent respectively On the other hand it must be admitted that this method of calculation may be misleading if the values are greatly reduced One can

scarcely speak of any noteworthy asymmetry if the extraction by one kidney is 10 and that by the other 20 per cent, whereas values of 30 and 60 per cent show a high degree of asymmetry

In the present series, the former circumstance plays little role as far as PAH extraction is concerned, since few patients had such a severe decrease as to 10 per cent However, with respect to renal concentration ability and the few determinations of *the glomerular filtration* rate, several patients had such low values that the percentage difference was extremely large, and might possibly give rise to misunderstanding, unless the mode of calculation is borne in mind

The functional capacity of the poorer kidney was used as a measure of progression of the disease as regards the extraction ability Obviously, in this connexion, no importance can be ascribed to the capacity of the better kidney since, theoretically, it can be only inappreciably involved irrespective of the degree of damage to the poorer kidney For the same reason, the mean of the values for the two kidneys cannot be regarded as a *measure of the extent of the* pathologic process It must, of course, be emphasized that the reduction in extraction ability alone was regarded as only one of many signs of the degree of severity of the disease

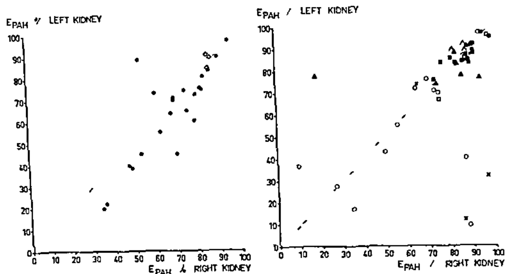
It can be inferred from Figure 2B that the degree of asymmetry had a distinct tendency to increase with an increasing severity of renal damage *This can be interpreted to* imply that when there is severe damage to the proximal tubules of the poorer kidney the two kidneys are usually involved to a different extent, whereas slight damage to these tubules is generally fairly symmetric This tendency is evident when the differences in E_{PAH} are plotted against the F_{PAH} of the poorer kidney, irrespective of whether these differences are expressed as percentages or numerically

It is however, apparent from Figure 2B that this relation also applied to groups B1—B5 (cases other than definite, chronic non obstructive pyelonephritis) As far as obviously unilateral renal diseases are concerned, this was to be expected, but it was also found in a few patients in groups B4 and B5, in whom there was no reason to anticipate asymmetric function

A noteworthy observation was made when studying which of the kidneys was more *involved in the presence of asymmetry* Figure 3A shows that in most cases this was the left kidney The mean value for extraction by the right kidney in the cases of chronic pyelonephritis was 71 12 ± 3 08 per cent ($n = 35$) and that by the left kidney 64 32 ± 3 54 per cent ($n = 35$) The difference 6 81 ± 2 07 per cent is highly significant ($p < 0\ 001$)

The implication of this finding is that in chronic pyelonephritis the proximal tubules of the left kidney are more susceptible to injury than the corresponding tubules of the right kidney

The suspicion might arise that the lower extraction value was actually due to the renal vein blood obtained through the catheter in the left kidney being mixed with blood from the suprarenal and the testicular or ovarian vein (*cf* Chap VIII) This suspicion is *however opposed by the fact that* the PAH clearance was also lower in the left kidney in 7 of 10 cases and higher in only 3 Another argument indicating that the extraction values represent a real functional difference is that the catheter was advanced far into the renal vein and its



A Chronic and acute non obstructive pyelonephritis (groups A1 A?)

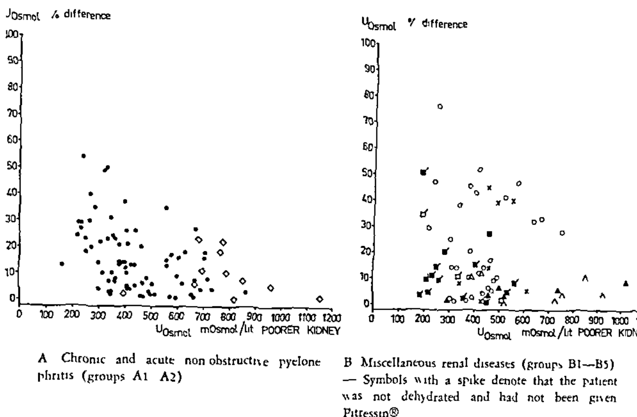
B Miscellaneous renal diseases (groups B1—B5)

Fig 3 PAH extraction (%) by the left kidney (ordinate) and the right (abscissa) — The broken line represents symmetric extraction For symbols see p 39

position carefully checked at each examination This diminished the risk of admixture with blood from the vena cava The fact that the right renal vein is normally shorter than the left introduces another source of error Thus when renal vein blood is withdrawn for determination of E_{PAH} there may be a greater risk of obtaining caval blood on the right side than on the left However if this side difference was of any importance it would have resulted in apparently lower extraction on the right side Consequently the difference noted cannot have been due to this source of error More over the contribution of blood from the veins opening into the renal vein must have been inappreciable even in cases in which the renal plasma flow was low (Table VIII) Finally if the admixture of blood from tributaries had affected the extraction by the left kidney this should have been noticeable in the determinations in healthy subjects as well which was not the case (Table III)

Bradley *et al* (25) also found that the left kidney generally had a lower PAH extraction than the right and ascribed this difference to the addition of blood from tributaries These authors case material consisted both of 22 subjects with healthy kidneys and of patients with such diseases as arterial hypertension and chronic glomerulonephritis Their explanation does not however seem to be adequate (*cf* Chap VIII)

No corresponding dominance of poorer PAH extraction by the left kidney was observed in groups B1—B5 (Fig 3B) These groups are not, however fully comparable since the great majority of patients had normal or only inappreciably reduced extraction In two of the three cases in which



A Chronic and acute non obstructive pyelonephritis (groups A1 A2)

B Miscellaneous renal diseases (groups B1—B5) — Symbols with a spike denote that the patient was not dehydrated and had not been given Pitressin®

Fig 4 Difference (%) between the concentration ability of the left and the right kidney (ordinate) and the concentration ability (mOsmol/lit) of the poorer kidney (abscissa) — For symbols see p 39

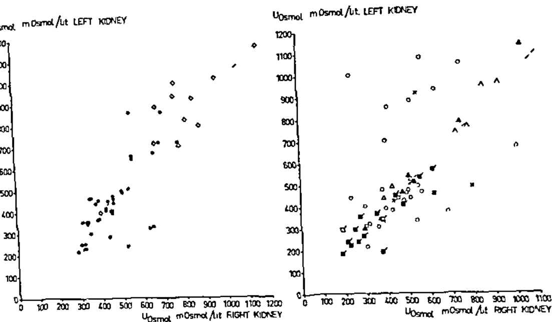
the greater decrease was noted for the left kidney the diagnosis was secondary chronic pyelonephritis and the third patient had hypoplasia of the left kidney No significant *difference was demonstrable between the* right and left kidney in this respect

Asymmetry of concentration ability

It can be inferred from Figure 4A (groups A1, A2) that with respect to renal concentration ability as well, there was a tendency to increasing asymmetry with an increasing degree of renal damage It must however be mentioned that some patients who had a good concentration ability in one kidney showed a fairly marked degree of asymmetry Thus in these cases, the better kidney had completely or almost completely unimpaired concentration ability Similar observations were made in groups B1—B5 (Fig 4B) A few patients with renal damage that could be expected to be homogeneous (*e g the nephrotic syndrome and chronic glome*rulonephritis) showed only inappreciable asymmetry of concentration ability

If a comparison is made between the concentration ability of the right and the left kidney (Fig 5A) the conditions differ distinctly from those in PAH extraction Although the left kidney showed poorer concentration ability than the right in a larger number of cases (in 42 and 56 determinations respectively) the mean difference was relatively small and was not significant

No tendency to a greater reduction in the concentration ability of the left kidney was



A Chronic and acute non-obstructive pyelonephritis (groups A1 A2)

B Miscellaneous renal diseases (groups B1—B5) — Symbols with a spike denote that the patient was not dehydrated and had not been given Pitressin®

Fig 5 Concentration ability (mOsmol/lit) of the left kidney (ordinate) and of the right (abcissa) — The broken line represents symmetric concentration ability For symbols see p 39

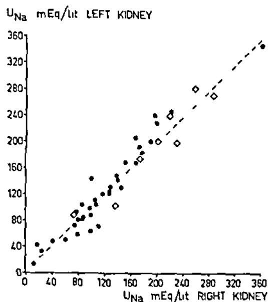
observed in groups B1—B5 (Fig 5B) It is evident from this figure that remarkably many patients had a symmetric reduction in this function, as can also be seen in Figure 4B

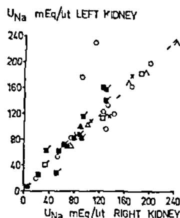
Asymmetry of sodium concentration

A comparison between the Na concentration in the urine from the right and the left kidney could have been expected to disclose approximately the same conditions as in the case of the osmolarity Figure 6A nevertheless shows that there was relatively little difference between the two kidneys in this respect In the cases of pyelonephritis (groups A1 A2) the scattering around the line of symmetry seemed in fact, even to be less than for the osmolarity (Fig 5A) The scattering was greater in groups B1—B5 (Fig 6B) but it must be noted that the cases which showed the greatest deviation were precisely those in which asymmetry could be anticipated (*e g* two cases of chronic pyelonephritis with nephrolithiasis and several with unilateral stenosis of the main renal artery) The latter patients were not deprived of fluids before the test

Asymmetry of endogenous creatinine inulin and PAH clearances and of RPF

In the few cases in which the creatinine and or inulin clearance by each of the kid

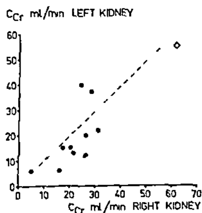


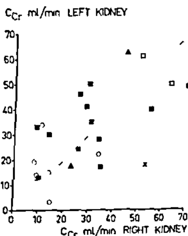


A Chronic and acute non obstructive pyelonephritis (groups A1 A2)

B Miscellaneous renal diseases (groups B1—B5) — Symbols with a spike deno e that the patient was not dehydrated and had not been given Pitressin®

Fig 6 Sodium concentration (mEq/lit) in urine from the left kidney (ordinate) and from the right (abscissa) — The broken line represents symmetric sodium concentration For symbols see p 39

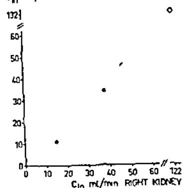


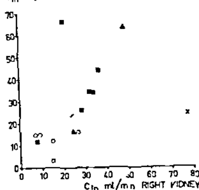


A Chronic and acute non obstructive pyelonephritis (groups A1 A2)

B Miscellaneous renal diseases (groups B1—B5)

Fig 7 Endogenous creatinine clearance (ml/min) by the left kidney (ordinate) and by the right (abscissa) — The broken line represents symmetric clearance For symbols see p 39

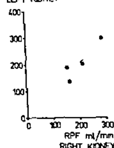


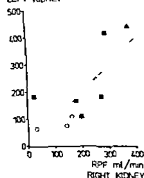


A Chronic and acute non-obstructive pyelonephritis (groups A1 A2)

B Miscellaneous renal diseases (groups B1—B5)

Fig 8 Inulin clearance (ml/min) by the left kidney (ordinate) and by the right (abscissa) — The broken line represents symmetric clearance For symbols see p 39





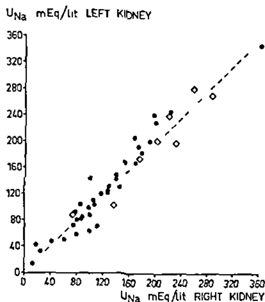
A Chronic and acute non-obstructive pyelonephritis (groups A1 A2)

B Miscellaneous renal diseases (groups B1—B5)

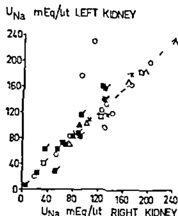
Fig 9 Renal plasma flow (ml/min) in the left kidney (ordinate) and in the right (abscissa) — The broken line represents symmetric RPF For symbols see p 39

neys could be determined the value for the left kidney was lower in most of those in groups A1 and A2 (Figs 7A 8A) Obviously the PAH clearance in itself is of little value in cases such as these with greatly impaired renal function and decreased PAH extraction The values have been included in *the tables only to illustrate the existence of* asymmetry However the difference is not significant so that no definite conclusions can be drawn As previously mentioned the PAH clearance was lower on the left side in 7 of 10 cases in groups A1 and A2

The RPF showed no preponderance of

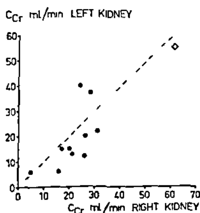


A Chronic and acute non obstructive pyelonephritis (groups A1 A2)

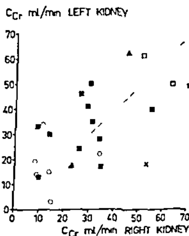


B Miscellaneous renal diseases (groups B1—B5) — Symbols with a spike denote that the patient was not dehydrated and had not been given Pitressin®

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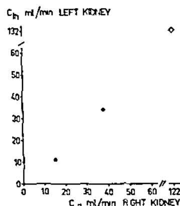


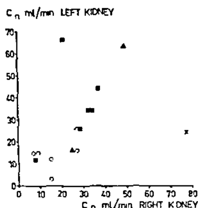
A Chronic and acute non-obstructive pyelonephritis (groups A1 A2)



B Miscellaneous renal diseases (groups B1—B5)

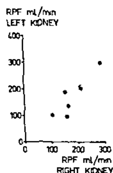
Fig 7 Endogenous creatinine clearance (ml/min) by the left kidney (ordinate) and by the right (abscissa) — The broken line represents symmetric clearance For symbols see p 39

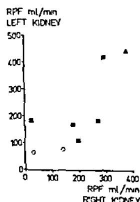




A Chronic and acute non-obstructive pyelonephritis (groups A1 A2)   B Miscellaneous renal diseases (groups B1—B5)

Fig 8 Inulin clearance (ml/min) by the left kidney (ordinate) and by the right (abscissa) — The broken line represents symmetric clearance For symbols see p 39





A Chronic and acute non-obstructive pyelonephritis (groups A1 A2)   B Miscellaneous renal diseases (groups B1—B5)

Fig 9 Renal plasma flow (ml/min) in the left kidney (ordinate) and in the right (abscissa) — The broken line represents symmetric RPF For symbols see p 39

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The RPF showed no preponderance of

either kidney in groups A1 and A2, on the contrary, these few values were surprisingly symmetric (Fig 9A)

The cases in groups B1—B5 were too *heterogeneous* to allow any uniform evaluation of the symmetry or asymmetry of the clearances in question, or of the RPF (Figs 7B, 8B, 9B) Moreover, the data obtained in groups B1—B5 were too few to permit any statistical analyses

Conclusions

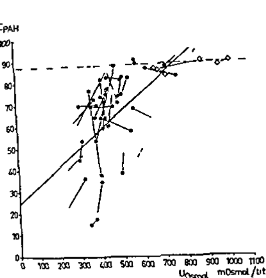
Any speculations on why, in chronic pyelonephritis, the proximal tubules of the left kidney seemed to have been more involved than those of the right (difference between the PAH extraction on the two sides) must, for the time being be limited to conjectures The left kidney is, as a rule, slightly larger than the right (*cf* Chap II) The pedicle of the former is, however, longer than that of the latter, which might increase the risk of kinking of the ureter and vessels with urinary stasis or circulatory disturbances as a result There is no reason to suspect that such kinking is more common in patients with chronic non obstructive pyelonephritis than in healthy subjects On the other hand such an impediment could naturally accentuate the effect of existing pyelonephritis and in this way affect the PAH extraction among other functions No other anatomic difference exists which might be responsible for a greater susceptibility of the left kidney to functional impairment

Another possible explanation is that the left kidney moves more than the right in connexion with respiration This was demonstrated, *e g* by Hilgenfeldt (99) by means of pyelography He stated that anatomists have found better fixation of the left kidney than of the right to the diaphragm This would allow it to follow the diaphragmatic excursions more closely Hilgenfeldt gave a detailed anatomic description, and stated that the kidney s capsule — particularly the inner layers of fat — provide better sliding movements than the outer layers

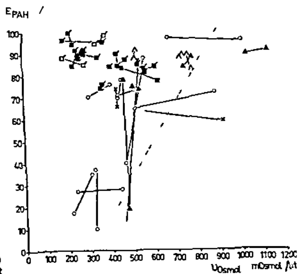
It seems plausible that this massage of the kidney favours the spreading of pyelonephritis, and perhaps also that the cortex is then involved to the greatest extent This would explain why the excretion by the proximal tubules in particular and thereby the PAH extraction as well are affected to a greater extent in the left kidney The same argument can be applied to the glomeruli This hypothesis is not however supported by any other experimental evidence or objective observations than the aforementioned Furthermore, Bacon (4) stated that he was unable to verify Hilgenfeldt s claim that the left kidney is more mobile than the right

B Relation Between the Types of Functional Damage and Their Pathophysiologic Implications in the Individual Kidney

Comparisons between the type and degree of damage to the individual renal functions may afford a possibility of drawing conclusions about the topography of development and progression of a pathologic process in the renal tubules If the extent of the process differs in the two kidneys examination of the bladder urine and of the extraction ratio in only one kidney may give misleading results



A Chronic and acute non obstructive pyelonephritis (groups A1 A2) — The linear regression line for E_{PAH} values below 85 % (—) follows the equation $y = 24.65 + 0.085\ x$ where y denotes E_{PAH} and x denotes U_{Osmol} in dehydration ($n = 45$) The equation for the regression line calculated on the cases with E_{PAH} above 85 % (— — —) is not significant ($n = 13$) cf p 99



B Miscellaneous renal diseases (groups B1—B5) — Symbols with a spike denote that the patient was not dehydrated and had not been given Pitressin®

Fig 10 PAH extraction ratio (% ordinate) and renal concentration ability (mOsmol/lit abscissa) — The values in the same patient are joined by a line The broken line represents the hypothetical position of the values if the extraction ratio and concentration ability had been depressed to the same degree For symbols see p 39

PAH extraction/concentration ability

Figure 10A shows the PAH extraction and the concentration ability of each kidney in relation to the contralateral one in the cases of chronic and acute non obstructive pyelonephritis (groups A1 A2) Each dot on the plot represents the PAH extraction by one kidney in relation to its concentration ability The values for the right and left kidney in each case are joined by a line Cases with the same value on the abscissa but a different one on the ordinate imply that the concentration ability is symmetric and the extraction asymmetric Cases with the same value on the ordinate and a different one on the abscissa denote the reverse

The fact that the dots for right and left kidney lie far from each other in most cases is already an indication that the functional impairment was usually asymmetric In 8 cases the asymmetry was slight as regards PAH extraction and in at least 12 it was slight as regards concentration ability Consequently the material does not permit the

either kidney in groups A1 and A2, on the contrary, these few values were surprisingly symmetric (Fig 9A)

The cases in groups B1—B5 were too heterogeneous to allow any uniform evaluation of the symmetry or asymmetry of the clearances in question, or of the RPF (Figs 7B, 8B, 9B) Moreover, the data obtained in groups B1—B5 were too few to permit any statistical analyses

Conclusions

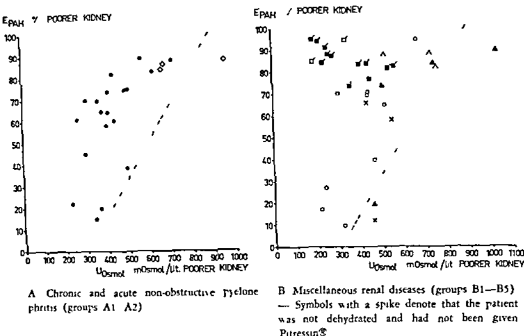
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Comparisons between the type and degree of damage to the individual renal functions may afford a possibility of drawing conclusions about the topography of development and progression of a pathologic process in the renal tubules If the extent of the process differs in the two kidneys examination of the bladder urine and of the extraction ratio in only one kidney may give misleading results



A Chronic and acute non-obstructive pyelonephritis (groups A1 A2)

B Miscellaneous renal diseases (groups B1—B5) — Symbols with a spike denote that the patient was not dehydrated and had not been given Pitressin®

Fig 11 PAH extraction ratio (%) of the kidney with the lower E_{PAH} (ordinate) and concentration ability (mOsmol/lit) of the kidney with the lower U_{Osmol} (abscissa) The broken line represents the hypothetical position of the values if the extraction ratio and concentration ability had been depressed to the same degree — For symbols see p 39

distal tubules to the same degree the plotted values should lie along the aforementioned straight line irrespective of the grade of severity of the damage

However if a comparison is made between e g the extraction and the concentration ability in the present series it is found that the values do not follow this ideal line (Fig 10A) Without exception they lie on that side of the ideal line which implies higher extraction than concentration ability This could be interpreted to mean that the extraction ability is relatively less reduced than the concentration ability This in turn would imply that the injury has been greater to the medulla where concentration takes place than to the cortex where extraction takes place This interpretation is compatible with the observation that in the cases with the lowest PAH extraction the concentration values approached the hypothetical origo This would denote that in severely damaged kidneys there is an almost complete lack of both extraction and concentration

The linear regression line for all the decreased E_{PAH} values (those below 85 per cent) in relation to U_{Osmol} followed the equation $y = 24.65 + 0.085\ x$ where y denotes E_{PAH} and x denotes U_{Osmol} in dehydration The slope of the line is highly significant ($t = 3.73$ $n = 45$) The equation for the regression line calculated on the cases with E_{PAH} above 85 per cent is not

conclusion that certain *parts of the tubules* have a greater tendency than others to an *asymmetric functional* impairment

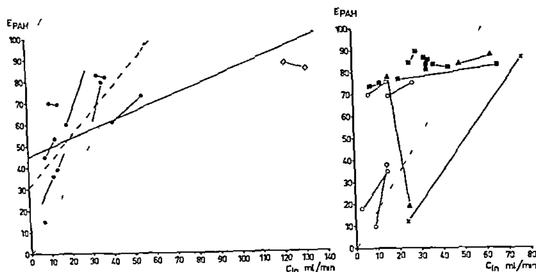
The results do, on the other hand, indicate that in chronic pyelonephritis the concentration ability is more severely and more often affected than is the extraction ability. It is evident from Figure 11A that several kidneys in the present material had exceedingly poor or almost lacking concentration ability, whereas the PAH extraction was only *slightly reduced — in some cases it* was even normal. (It must be emphasized *that the PAH* concentration in the arterial blood was low in every case.) In no case did the reverse apply, *i e*, poor extraction with normal concentration ability.

This observation might be explained in two ways. One is that the renal medulla (Henle's loop, the collecting ducts and the medullary circulation) is more severely damaged by the disease. The other is that *it is more sensitive to the effects of the* disease. Since it is probable that pyelonephritis frequently progresses in the retrograde direction, the former explanation appears more likely. Proof of this assumption requires comparative studies in other forms of renal disease. A small series of this kind is presented in Figure 10B but it is unfortunately too small and too heterogeneous to offer any convincing proof. Nevertheless in these few cases as well — some of which were secondary pyelonephritis — there was a lack of parallelism between the extraction and concentration abilities. Any definite conclusion must await a large number of *cases*.

It can be postulated that the PAH extraction is a *measure of the functional* capability of the proximal tubules (59), the concentration ability a *measure of the function* of the *collecting* tubules, and the creatinine or inulin clearance a measure of the glomerular filtration. These parameters could then be used to compare the degree of damage to the different parts of the nephron. If they are plotted against each other in a coordinate system the origo should lie at approximately 300 mOsmol/lit (isostenuria) for the concentration ability, slightly above 0 for the extraction ability (due *to the PAH filtered in* the glomeruli) and at 0 for the glomerular *filtration rate. If all parts of the nephron* were invariably damaged to the same degree the values should lie on a line joining this origo with a point corresponding approximately to 85 per cent for the extraction ability, 800 mOsmol/lit for the concentration ability, and 50 ml/min for the glomerular filtration rate.

It is doubtful whether such a quantitative calculation of the degree of damage is justified. In the first place the normal maximum *capacity of the individual patient's* renal function cannot be established exactly. The *aforegoing values are therefore estimates* based on the normal values given in the literature and the average age *of the patients*. Moreover it naturally cannot be claimed, *e g* that the damage to the collecting tubules is twice as great when the concentration ability is 400 mOsmol/lit as when it is 500. Nor can it be stated that when the extraction by the proximal tubules is 30 per cent they are twice as much damaged as when the corresponding figure is 60 per cent. Thus the comparison was made with due reservation for the large sources of error inherent in the hypothesis. I nevertheless considered it to be of some interest for a study of the agreement or lack of agreement in the general relation between the tests.

If renal damage involves proximal and



A Chronic and acute non-obstructive pyelonephritis (groups A1–A?) — The linear regression line for all values (—) follows the equation $y = 45.17 + 0.41\,x$ where y denotes E_{PAH} and x denotes C_I ($n = 20$). The linear regression line for all values except those in acute pyelonephritis (— — —) follows the equation $y = 30.93 + 1.13\,x$ ($n = 18$) cf. p. 100

B Miscellaneous renal diseases (groups B1—B5)

Fig. 13 Renal extraction of PAH (% ordinate) and inulin clearance (ml/min abscissa) — The values in the same patient are joined by a line. The broken line represents the hypothetical position of the values if the extraction rate and inulin clearance had been depressed to the same degree. For symbols see p. 39

in the same direction for both these parameters in most cases of chronic and acute non-obstructive pyelonephritis (Fig. 13A). No definite correlation seemed on the other hand to be present between the size of the percentage differences for concentration ability *versus* Na concentration. In some cases the difference was even reversed, *i.e.* the kidney which concentrated more effectively produced urine with a lower Na concentration than the other. Arterial hypertension (B.P. 180/110 mm Hg) was present in only one of these 8 slightly deviating cases (case 32). Renal angiography was not however performed in any of these patients.

The aforementioned discrepancy was still more conspicuous in the cases of other renal diseases (Fig. 13B). It is striking to note that several cases of renal artery stenosis in particular showed this inverse relation. A similar observation has been reported earlier (*e.g.* 134).

It is difficult to find any explanation of the strange circumstance that the kidney with a poorer blood supply excretes urine containing more solutes and less sodium than that

significant ($t = 0.32$, $n = 13$) However, both these lines show a highly significant deviation from the hypothetical ideal line ($t = 4.50$ and 5.67, respectively)

Returning to the few cases of other renal diseases, a tendency can, it is true, be seen to a displacement of the values to that side of the ideal line implying that concentration is relatively more impaired than extraction (Fig 10B) In cases with values in this position, it can be postulated that the damage has involved the collecting and the proximal tubules to approximately the same extent It must, however, be emphasized that the series is far too small to permit any definite conclusions

PAH extraction/inulin or endogenous creatinine clearance

When comparing the PAH extraction with the inulin or endogenous creatinine clearance the large sources of error that may be associated with unilateral clearance determinations must be borne in mind (*cf* Chap VIII) It was, however, found that reasons exist for believing that such errors were of relatively little consequence in the present series

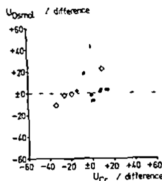
With reservation for these sources of error it is seen that the values for PAH extraction were also better than those for inulin clearance (Fig 12A) In the patients with pyelonephritis, the linear regression line for E_{PAH} in relation to C_{In} followed the equation $y = 45.17 + 0.41x$, where y denotes E_{PAH} and x denotes C_{In} The slope of the line is significant ($t = 3.53$ $n = 20$), and shows a highly significant deviation from the hypothetical ideal line ($t = 10.50$) It is, however highly questionable whether this regression line does, in fact represent the conditions in chronic pyelonephritis This is because, if the only case of acute pyelonephritis in this plot is excluded, the regression line would have a completely different course (equation $y = 30.93 + 1.13x$ The slope of this line is then significant ($t = 4.20$, $n = 18$), but does not differ significantly from the hypothetical ideal line ($t = 2.04$, $n = 18$) If this represents the true conditions, it indicates that in chronic pyelonephritis the proximal tubules are generally less damaged than the glomeruli It must, however, be pointed out that if the error due to leakage during collection of the urine takes effect in clearance determinations it would result precisely in such a shift of the values from the ideal line as that shown in Figure 12A

My observation — confined to a few cases — is not in conformity with the results of Raaschou (200) and Kleeman *et al* (135) who found that the GFR was preserved to a greater extent than the function of the proximal tubules (T_{mD} T_{mPAH}) Michie & Michie (163) demonstrated on the contrary that GFR and T_{mPAH} decreased proportionately in cases of chronic pyelonephritis

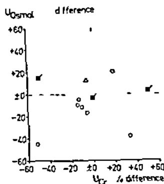
In the few cases of renal diseases other than chronic and acute non obstructive pyelonephritis there was a still greater tendency to retention of the extraction function even with greatly decreased GFR (Fig 12B) A comparison between PAH extraction and endogenous creatinine clearance gave similar results

Concentration ability/Na concentration

The percentage difference between the kidneys with respect to the urinary concentration of Na and the corresponding difference in concentration ability were also compared As could be expected there was a difference



A Chronic and acute non-obstructive pyelonephritis (groups A1 A2)



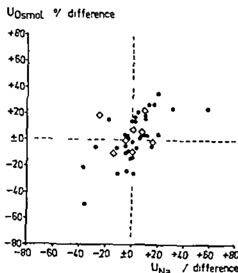
B Miscellaneous renal diseases (groups B1—B5) — Symbols with a spike denote that the patient was not dehydrated and had not been given Pitressin®

Fig. 14 Difference (%) between the concentration ability of the left and the right kidney (ordinate) and the corresponding difference (%) in urinary creatinine concentration (abscissa) — A plus sign before the numeral denotes that the value for the right kidney is higher than that for the left, and a minus sign *vice versa* The broken lines represent symmetry For symbols see p 39

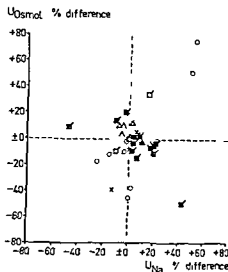
Concentration ability/inulin clearance

In a comparison between the concentration ability and inulin clearance it is found that all the patients in whom both tests were made had fairly or greatly reduced concentration ability but that the glomerular filtration rate showed a wide range of variation (Fig. 15A) This indicates that Henle's loop and the collecting tubules are — or may be — more severely damaged than the glomeruli whereas the reverse did not apply in any of my cases The linear regression line for C_{In} in relation to U_{Osmol} followed the equation $y = 280.77 + 3.16x$ where y denotes U_{Osmol} in dehydration and x denotes C_{In} The slope of the line is highly significant ($t = 7.46$ $n = 20$) as is its deviation from the hypothetical ideal line ($t = 16.19$)

This dissociation of function has been pointed out earlier by Brod (28 29) and by Winberg (269) in acute pyelonephritis Brod stated that it is an extremely useful diagnostic sign in all developmental phases of pyelonephritis as well as for a differential diagnosis between chronic pyelonephritis and nephrosclerosis In Figure 15B the osmolarity of the urine is plotted against the inulin clearance in patients belonging to groups B1—B5 most of whom had essential hypertension Here the values lay closer to the ideal line and in most cases the functional impairment was parallel in both parameters in each of the kidneys This is in agreement with Brod's results (29) In his series he found that the regression lines for chronic non obstructive pyelonephritis chronic glomerulonephritis and nephrosclerosis lay above each other in that order with the line for pyelonephritis closest to the x axis (GFR) Bengtsson (10b) on the other hand noted no discrepancy between GFR and renal concentration ability in her series of chronic pyelonephritis In another of her



A Chronic and acute non obstructive pyelonephritis (groups A1 A2)



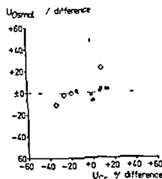
B Miscellaneous renal diseases (groups B1—B5) — *Symbols with a spike denote that the patient was not dehydrated and had not been given Pitressin®*

Fig 13 Difference (%) between the concentration ability of the left and the right kidney (ordinate) and the corresponding difference (%) in urinary sodium concentration (abscissa) — A plus sign before the numeral denotes that the value for the right kidney is higher than that for the left and a minus sign *vice versa* The broken lines represent symmetry For symbols see p 39

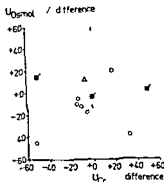
with a normal blood flow Since it is known that the stenosed kidney also has a smaller urinary output (Howard s test) it is probable that the reabsorption of water as well is greater on this side (unless the glomerular filtration is considerably reduced) If this reabsorption is not due to the ADH but is more of the isotonic type, the concentration of urea and other osmotic substances would increase with decreasing Na concentration It must however be pointed out that the osmolality of the urine was determined with out the patients being dehydrated, which diminishes the value of the comparison

Concentration ability/urinary creatinine concentration

Since, in a dehydrated subject a difference between the concentration ability of the right and left kidney is in all probability due to a difference in the reabsorption of water one can expect a corresponding difference in the urinary concentration of creatinine Figure 14A shows the relation between asymmetry of concentration ability and the corresponding asymmetry of creatinine concentration It can be seen that in the majority of cases the asymmetry of these two parameters of water reabsorption ran parallel The range of the values was however too wide to warrant any attempt at calculating the regression or correlation The reason for this relatively wide range is probably that the creatinine concentration is a function of the total reabsorption of water *i e* of the proximal tubules as well whereas the osmolarity is not regulated until the urine reaches the collecting ducts



A Chronic and acute non-obstructive pyelonephritis (groups A1 A2)



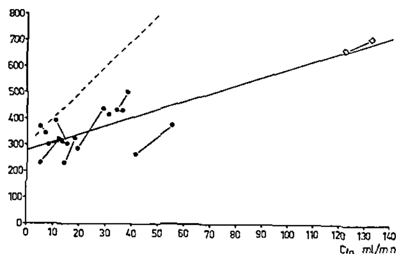
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Fig 14 Difference (%) between the concentration ability of the left and the right kidney (ordinate) and the corresponding difference (%) in urinary creatinine concentration (abscissa) — A plus sign before the numeral denotes that the value for the right kidney is higher than that for the left, and a minus sign *vice versa* The broken lines represent symmetry For symbols see p 39

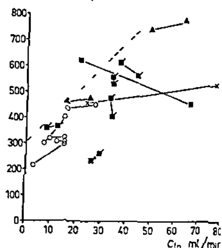
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B Miscellaneous renal diseases (groups B1—B5) — Symbols with a spike denote that the patient was not dehydrated and had not been given Pitressin®

Fig 15 Renal concentration ability (mOsmol/lit ordinate) and inulin clearance (ml/min abscissa) — The values in the same patient are joined by a line The broken line represents the hypothetical position of the values if the concentration ability and inulin clearance had been depressed to the same degree For symbols see p 39

groups — consisting of cases of chronic renal papillitis — the renal concentration ability was on the contrary more reduced than the GFR She concluded that this classification of the material and exclusion of cases of *acute pyelonephritis* were responsible for the discrepancy between her latest results (10b) and her previous ones (10a) and between hers and those of Brod (29) Raaschou (200) also found that there was on the whole, a correlation between renal concentration ability and GFR (measured as the inulin and urea clearance)

A greater reduction in renal concentration ability *than in GFR has been demonstrated* in hydronephrosis — also without infection — in man (18, 269) as well as in acute obstruction of the urinary tract in dogs (114) Two of these authors made separate bilateral studies of renal function (114 269) My series contained only two patients with unilateral hydronephrosis (cases 109 and 110) Since the renal concentration test was not made in either of them no comparison can *be made between the aforementioned functions*

Potassium deficiency (159 207) like hypercalcaemia (74 160) can also produce a greater reduction in renal concentration ability than in GFR In these disorders the damage to the renal medulla seems to be caused by a similar mechanism (74) My series contained only one patient with potassium deficiency *(case 19), but three with hypercalcaemia* In two of the latter hypercalcaemia was due to hyperparathyroidism (cases 105 and 109) and in the remaining one *to renal tubular acidosis* (case 115) The renal concen

tration test was not made in case 109 (*cf* hydronephrosis) The other two patients with hypercalcaemia showed a greater reduction in concentration ability than in GFR which is in agreement with the aforementioned authors observations

Concentration ability PAH extraction endogenous creatinine clearance and RPF

Edvall (68) compared the E_{PAH} and RPF in urological cases and found good agreement between them In his series the effective RPF was determined as the PAH clearance in bladder urine whereas E_{PAH} was determined in the urine from one kidney In my material the RPF was plotted on one axis and the renal extraction ability and concentration ability respectively on the other However since no relation seemed to be present between these parameters the graphic representation of the results has been omitted On the other hand — as could be expected — a definite relation was present between RPF and endogenous creatinine clearance as demonstrated previously (135)

Conclusions

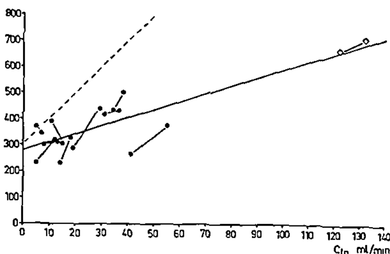
If any importance is to be ascribed to the comparisons presented in the aforegoing as a quantitative measure of the relevant degree of damage they imply that the concentrating part of the nephron (particularly Henle s loop and the collecting tubules) is most severely involved in chronic pyelonephritis The glomeruli are next in order with respect to the degree of damage whereas the proximal tubules seem as a rule to be less involved In other words the results indicate that in chronic pyelonephritis, the renal medulla is more severely damaged than the cortex

It is not until the renal damage has reached an extremely high degree — so that the function of all parts of the nephron is close to a minimum — that some parallelism is observed between the functional impairment of the various parts of the nephron, *i e* the values approach the origo on the plots Obviously in subjects with completely healthy kidneys fairly good agreement would be present, since the peak of the ideal curve is plotted from the normal functional values It is evident that there will be wide scattering of the values around this peak in view of the large variations which exist in renal concentration ability and glomerular filtration rate

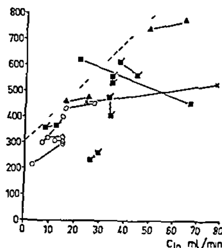
As stated earlier the values in my patients belonging to group A2 (*i e* chiefly the cases of acute pyelonephritis) were included in the plots despite the uncertainty of the diagnosis These results were concentrated around the upper part of the line which denotes a similar functional impairment — thus close to the values in healthy subjects Most of these cases would then represent those in which renal damage was inappreciable

The *highest diastolic pressure* of several recordings over 24 hours was plotted against the E_{PAH} of the poorer kidney as well as against U_{Osmol} after withholding fluids In groups A1 and A2 no correlation was demonstrable in either respect

The results of the functional tests described in the aforegoing are thus largely in agreement with the observations in animal experiments showing that the renal medulla is usually more sensitive to infection than the cortex in experimental chronic pyelonephritis (2 80 81 89 212 213) In attempts to induce pyelonephritis in animals by local injection of bacteria at various sites



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B Miscellaneous renal diseases (groups B1—B5) — Symbols with a spike denote that the patient was *not dehydrated* and had not been given Pitressin®

Fig 15 Renal concentration ability (mOsmol/lit ordinate) and inulin clearance (ml/min abscissa) — The values in the same patient are joined by a line The broken line represents the hypothetical position of the values if the concentration ability and inulin clearance had been depressed to the same degree For symbols see p 39

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termining the nature of any renal damage Their method was subsequently refined (15) It was based on the premise that a reduced extraction of PAH was to be ascribed essentially to two different causes *i e*

1 Most PAH excreting tubules were involved to some extent by the renal damage and

2 Only a limited number of tubules were damaged whereas others extracted PAH to the normal extent

Smith and his co workers (232 234 236 237) had shown by means of so-called titration experiments that if the concentration of PAH in plasma was gradually raised from low to increasingly high levels the tubular excretion of PAH initially rose in proportion to the tubular load of PAH (renal plasma flow × plasma PAH concentration) However when the plasma concentration exceeded a certain limit (saturation limit) the tubular excretion ceased to rise It remained stable despite a rising plasma concentration This implied that the tubules had reached their maximal excretory rate Tm_{PAH}

Josephson *et al* (119) and Bergstrom *et al* (15) tried to achieve corresponding results by determining the renal extraction of PAH instead of T_m which is hard to determine They reasoned that if the PAH concentration in plasma was gradually raised from low values the renal extraction should remain unchanged as long as no tubule had reached its T_m This implied in other words for as long as the capacity of the excretory apparatus sufficed to deal with all the PAH delivered to it by the blood The renal extraction should not start to fall until the concentration had reached T_m (the self depression limit) Furthermore this fall should run parallel to the rise in PAH concentration since the kidneys would not be able to extract from the blood more PAH than that represented by T_m plus the glomerular filtration The saturation limit thus denotes the PAH concentration at which all the tubules had reached their maximal excretory capacity

It was also presumed (119) that if some of the proximal tubules excreted normally their self depression limit should lie on a normal level (11—13 mg/100 ml plasma) If at a low PAH concentration the extraction was reduced because the damaged tubules no longer excreted a rise in PAH concentration should not cause a further reduction in the total extraction until the maximal excretory capacity of the remaining normal tubules was reached — *i e* the self depression limit should be normal This would be ensured by the remaining healthy nephrons

If on the other hand all proximal tubules were damaged — so that their excretory capacity was more generally reduced — decreased extraction should be observed even when the PAH concentration in plasma was inappreciably raised

It can be pointed out that this determination does not require the urine to be measured or analyzed In tests with a gradually rising PAH concentration in plasma (so called staircase experiments) Josephson *et al* (119) and Bergström *et al* (15) did in fact in many cases note results which corresponded to their hypothesis

The results were however often uncertain This was partly because few cases of renal disease are actually so distinctly delimited that they give clear-cut results regarding the plasma concentration of PAH at which self depression of extraction occurs Moreover it was difficult to administer the PAH infusion in such a way that the intended step

in the renal tissues Andriole & Epstein (2) also found the medulla to be more susceptible to the disease than the cortex On the basis of earlier experience, the authors ascribed this to the higher osmolarity of the medullary tissues By giving the animals large quantities of water, they were able to keep the electrolyte concentration in the medulla on a low level, and thereby to decrease the incidence of pyelonephritis after infection Thus if pyelonephritis involves in the first place the renal medulla, the concentration ability should be more affected than the filtration and extraction abilities (74)

According to Beeson & Rowley (9) the chemical composition of the medullary tissues — particularly their content of ammonia and ammonia producing enzymes, which hamper complement activity — might also contribute to the greater vulnerability of this part of the kidney

The oldest explanation of a greater and earlier functional impairment of those parts of the nephron which are situated in the medulla is their anatomic localization in relation to the renal pelvis (55) This explanation appears highly probable at any rate in pyelonephritis caused by ascending infection

When comparing the occurrence of pathologic symptoms in the different parts of the nephron it is also necessary to bear in mind that the blood supply of the medulla is much smaller than that of the cortex It is considered that only a small percentage of the blood passing through the kidneys flows through the medulla whereas the greater part is received by the cortex (141 247 248)

Moreover, the oxygen supply of the medulla is also limited by the fact that oxygen is able to pass by diffusion from the descending to the ascending loop of the arteriolae rectae, which implies that the renal papillae are susceptible to hypoxia (255) Ulfendahl (255) showed in addition in the same investigation that the oxygen tension is much higher in the renal cortex than in the medulla According to Trueta *et al* (252), the blood flow through the kidneys can be partly shunted between the cortex and the medullary tissue It has also been stated that the vascular system of the cortex and that of the medulla can act relatively independently (256) It is probable that these *factors can combine to lower the resistance* of the renal medulla to an infective agent

The present results show that in chronic non obstructive pyelonephritis in man as well, the function of the distal parts of the nephron is more depressed than that of the proximal ones However because of the way in which the disease develops, this does not apply in extremely slight cases nor in highly advanced ones

C Interpretation of a Decreased Extraction Ability

It has long been known that in persons with healthy kidneys about 90 per cent of the plasma PAH content is extracted during passage of the blood through the kidneys provided that the plasma PAH concentration is kept below the so called self depression limit (119) In healthy subjects this limit is considered to range from 11—13 mg/100 ml plasma If the concentration is raised above this limit the extraction starts to fall and continues to do so if the concentration continues to rise

Josephson *et al* (119) found in a study of the PAH accumulation in the kidneys of man that the value for the self depression limit might be of some importance for de

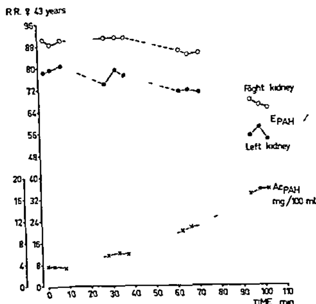


Fig 17 Extraction ratio (%) of PAH (o) and arterial PAH concentration (x) in a staircase experiment (case 92) — I v infusion started 5 min before zero time Infusion rate increased at 17 40 and 74 min after zero time

has therefore been used with advantage as a check on the PAH values It must however be emphasized that the extraction of Diodrast is determined in whole blood whereas that of PAH is determined in plasma This difference contributes to the discrepancies in extraction which may be observed

The results of these determinations are best illustrated graphically For reasons of space only two cases will be described

CASE 3 a 41 year-old woman (Fig 16) Diagnosis chronic pyelonephritis She had a history of recurrent cystitis since childhood Her blood pressure was normal as were the ocular fundi The bladder urine contained relatively numerous W B C and a growth of *E coli* PAH clearance 616 ml min Endogenous creatinine clearance 93 ml min Inulin clearance 115 ml/min Serum creatinine normal Renal biopsy acute to subacute interstitial nephritis Pyelography showed that the right kidney was smaller than the left and had a greatly decreased density of contrast medium dysplasia was present in its lower pole Determinations on catheter specimens from the ureters disclosed better function of the left kidney with respect to concentration ability as well as Na and creatinine concentration (Table X)

It can be inferred from Figure 16 that when the PAH concentration in plasma rose above 9 mg/100 ml, there was no measurable effect on the PAH extraction in the left kidney and that of Diodrast fell only slightly whereas both extraction values fell markedly in the right kidney At a low PAH concentration the left kidney showed normal extraction and the right an extremely slight reduction It therefore seemed reasonable to presume the existence of slight focal damage to the right kidney whereas the left appeared to be undamaged

CASE 92 a 43 year-old woman (Fig 17) Diagnosis chronic pyelonephritis + renal papillary necrosis During the past 6 years she had repeated attacks of cystitis accompanied by rigors and high fever She also had attacks of renal colic with passage of concretions (renal papillae?) Her blood pressure was normal as were the ocular

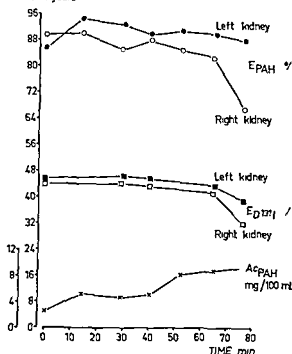


Fig 16 Extraction ratios (%) of PAH (o) and radioactive Diodrast ¹³¹I (□) and arterial PAH concentration (x) in a staircase experiment (case 23) — I v infusion of PAH and Diodrast started 83 min before zero time Infusion rate increased at 2 and 41 min after zero time

wise rise in plasma concentration was achieved Obviously, if the whole kidney is involved by a pathologic process, a certain number of normally functioning nephrons usually remain On the other hand one fraction of a majority of normal nephrons can be envisaged to be affected by its nearness to pathologic processes *e g* such in which toxic products are released Furthermore PAH can — at any rate under certain conditions — be accumulated in the renal tissue (122) This may result in increased extraction as long as the accumulation is in progress (119) and a decreased extraction value on release of the accumulated PAH

Another complicating factor is the risk of technical errors in the course of the determination For example, the position of the catheter tip may become altered during a long test, so that blood from the vena cava is mixed with that from the renal vein, or the patient may be affected *e g* by fatigue (*cf* Chap VIII)

Despite these limitations of the method I considered it of interest to test it in some cases in which the diagnosis of chronic pyelonephritis was regarded as unquestionable The chief reasons for selecting patients precisely with *chronic pyelonephritis* were the often focal nature of the disease and the fact that it not infrequently involves only certain nephrons in a bundle (27) I made such staircase experiments in 14 cases Since the object of the present investigation was to study the symmetry (or asymmetry) of renal function only brief mention will be made of some cases in which staircase experiments were made simultaneously on both kidneys

In some of them a sufficiently high PAH concentration was never attained (which was not detected until the chemical analysis was made) In others, jumping extraction values were recorded In five cases I succeeded in keeping a catheter in both renal veins during the rise in plasma PAH concentration so that the self depression limit of each of the kidneys could be studied simultaneously Some patients were given radioactive Diodrast in the same infusion solution as PAH This is because it has been shown that PAH and Diodrast are excreted by the same transport system or at any rate that they have some step in the transport system in common (*cf* Chap VII) If PAH and radioactive Diodrast are administered concurrently an increase in the plasma concentration of one of the substances has approximately the same depressive effect on the renal extraction of both substances (122) Radioactive Diodrast

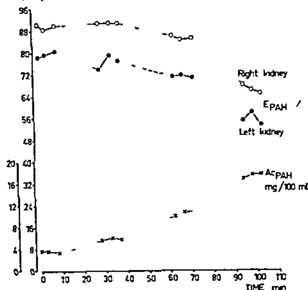


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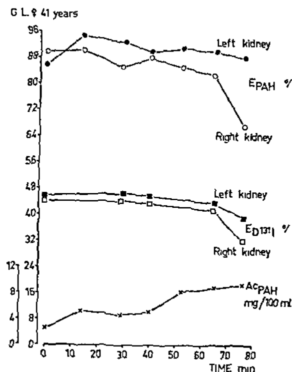


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Other spl t funct on tests such as T_mPAH $T_{m\ lucose}$ product on of ammon a and renal ac d fy ng ab l ty requ re load ng arrangem nts that are d fficult or mposs ble to ach e e in each kidney separately if the af rement oned funct ons are to be stud ed concurrently

The asymmetry of the arter o venous oxygen d fference has also been stud ed n various cl n cal cond tions (181)

As nd cated by Tables X XI and XII most renal funct ons proved to be asymmetr c in those cas s whe e asymmetry was expected n s far as any conclus ons can be drawn from the scanty data n som of the groups It s also seen that in e ery group there were certa n cases in wh ch symmetry b tween the k dneys was not d for som funct on th s appl d to all the parameters tested In some ca es t was d ff cult to dec de whether asymmetry d d n fact ex st s nce the r ght k dney p eponderated n one respect and the left n an ther D ergent asymmetry of th s k nd was relat ely common as regards the results of PAH extract on n pat ents w th chron c non obstructi e pyelonephr t s D ergent asymm try was also found n a few as s w th respect to tenderness to palpat on U_{Na} nd anamnest c data A noeworthy f atu e s that m st of these cases belonged to gr ups A1 A2 and B1 (chron c and acut non obstruct e and obs ruct e pyelo n ph t s) A poss ble explanat on of the d ergen e s that th s d sease can always be rega d d as b lateral consequently one or o h of the k dneys w ll dom nate for d f feren funct ns and symptoms at d fferent t mes D e gent asymmetry was ra e n e p of ro n genolog c exam nat on U_{Osmo} U_{Cr} RPF and GFR

Symmetr c renal fun t on was nd cated by some t s s n all groups The endogenous creat n ne clearance ind cated d vergent asymme ry only in one case n group A1 S nce the creat n ne clearance was d t rm ned in more cases than the nul n clearance evaluat n of asymmetry of GFR was based on the former dete m nat on

It cannot be concluded from the present study that the presence of asymmetry is a suff cient reason for mak ng a part cular d agnos s It can however be stated that asymmetry with respect to concentrat on ab l ty and U_{Cr} was more common in the cases of pyelon phr t s (groups A1 A2 and B1) than in th other groups but that no such d fference could be demonstrated for PAH extract on roentgenolog c exam na t on U_{Na} or GFR

Occurrence of Asymmetr c Funct on n Certa n Renal D seases

Chro c a d acute pyelo phr t s (groups A1 A2 and B1)

Asymmetr c renal funct on w th respect to a numb r of param ters has previously b en reported n cases of acute and chron c pyelonephr t s Some of these references are l sted n Table II

In chron c pyelonephr t s it must always be assum d that both k dneys are invol ed th s has been conf rmed by *e g* Brod (28) Raaschou (200) and Sensenbach (229) All seven cas s of chron c pyelonephr t s in the present ser es n wh ch autopsy was per form d had contracted k dneys (cases 5 19 28 35 39 83 90) No substant al d ffe rence could be detected between the two kid neys w th respect to weight and s ze presum ably b caus they were in an ad anced stage o anatom c destruct on The same trend appl ed to funct on Irrespect ve of the aet o logy some of the pat ents w th chron c pye lonephrit s had ad anced renal nsuff c ency

fundi PAH clearance 509 ml/min Endogenous creatinine clearance 81 ml/min The serum creatinine calcium and phosphate phosphorus concentrations were normal Renal biopsy focal interstitial inflammation as in pyelonephritis Pyelography showed that both kidneys were of the same size but that the parenchyma of the left was reduced and all its papillae had been sloughed Similar but less marked lesions were present in the right kidney Determinations on catheter specimens showed a moderate number of W B C and R B C as well as a growth of *E coli* (70 000/ml) in the urine from the left ureter but not in that from the right With respect to concentration ability and pH of the urine as well as creatinine concentration the left kidney showed poorer function than the right (Table X)

Figure 17 shows normal extraction by the right kidney with a self depression limit of about 11 mg/100 ml The left kidney had a lower extraction ratio irrespective of the PAH concentration The self depression limit was identical to that on the right side In this case, the results suggest focal damage to the left kidney

The results in these two cases illustrate that the staircase method may in some cases give additional information about the type and extent of involvement of the kidneys Despite some uncertainty the method may be of value for estimating the state of the excretory apparatus *i e* the proximal tubules This applies especially in a study of the possible asymmetry of function of this apparatus Obviously the method is too circumstantial and the procedure too difficult for it to have any routine clinical applicability

D Clinical Value of Testing for Asymmetry of Renal Function

Catheterization of the ureters or renal veins does not involve much discomfort to the patient and neither measure is risky or difficult to perform for anyone with some experience Both procedures are, however somewhat lengthy, particularly when combined with a functional diagnosis of the type described here Moreover, they require the assistance of trained staff special premises and — at any rate as far as renal vein catheterization is concerned — expensive equipment It is beyond the scope of the present study to attempt an assessment of the clinical value of ureteric and renal vein catheterization in general It nevertheless seems motivated to try to estimate the value — and in particular the clinical advantages — of ascertaining the presence of any asymmetry of renal function with these procedures above all in cases in which a definite diagnosis cannot be made with simpler methods Since chronic pyelonephritis is the disease (or group of diseases) constituting the majority of the cases in the present study the following discussion is primarily related to this complaint although other forms of renal diseases are also considered

Methods for Studies of Each Kidney Separately

As previously stated, the clearance of inulin and endogenous creatinine has been used to assess the glomerular filtration rate the PAH extraction to assess the function of the proximal tubules and the renal concentration ability to express the function in the distal parts of the nephron The creatinine concentration in the urine has been taken as an approximate measure of water reabsorption

In the present study the PAH extraction and the concentration ability were mainly used as parameters of renal function for the reasons given on page 88

Other split function tests such as T_mPAH $T_{m\text{glucose}}$ production of ammonia and renal acidifying ability require loading arrangem nts that a e difficult or impossible to achieve in each kidney separately if the aforementioned functions are to be studied concurrently

The asymmetry of the arterio-venous oxygen difference has also been studied in various clinical conditions (181)

As indicated by Tables X XI and XII most renal functions proved to be asymmetric in those cas s where asymmetry was expected in so far as any conclusions can be drawn from the scanty data in some of the groups It is also seen that in every group there were certain cases in which symmetry b tween the kidneys was noted for some function this applied to all the parameters tested In some cases it was difficult to decide whether asymmetry did in fact exist since the right kidney preponderated in one respect and the left in an ther Divergent asymmetry of this kind was relatively common as regards the results of PAH extraction in patients with chronic non obstructive pyelonephritis Divergent asymmetry was also found in a few cas s with respect to tenderness to palpation U_{Na} and anamnestic data A noteworthy featu e is that most of these cases belonged to gr ups A1 A2 and B1 (chronic and acute non obstructive and obstructive pyelonephritis) A possible explanation of the divergence is that this disease can always be regarded as bilateral consequently one or other of the kidneys will dominate for different functions and symptoms at different times Divergent asymmetry was ra e in re p c of roentgenologic examination U_{Osmol} U_{Cr} RPF and GFR

Symmetric renal function was indicated by some tests in all groups The endogenous creatinine clearance indicated divergent asymmetry only in one case in group A1 Since the creatinine clearance was determined in more cases than the inulin clearance evaluation of asymmetry of GFR was based on the former dete mination

It cannot be concluded from the present study that the presence of asymmetry is a sufficient reason for making a particular diagnosis It can however be stated that asymmetry with respect to concentration ability and U_{Cr} was more common in the cases of pyelonephritis (groups A1 A2 and B1) than in the other groups but that no such difference could be demonstrated for PAH extraction roentgenologic examination U_{Na} or GFR

Occurrence of Asymmetric Function in Certain Renal Diseases

Chron c and acute pyelon phritis (groups A1 A2 and B1)

Asymmetric renal function with respect to a number of parameters has previously been reported in cases of acute and chronic pyelonephritis Some of these references are listed in Table II

In chronic pyelonephritis it must always be assumed that both kidneys are involved this has b en confirmed by *e g* Brod (28) Raaschou (200) and Sensenbach (229) All seven cases of chronic pyelonephritis in the present series in which autopsy was performed had contracted kidneys (cases 5 19 28 35 39 83 90) No substantial difference could be detected between the two kidneys with respect to weight and size presumably because they were in an advanced stage of anatomic destruction The same trend applied to function Irrespective of the aetiology some of the patients with chronic pyelonephritis had advanced renal insufficiency

fundi PAH clearance 509 ml/min Endogenous creatinine clearance 81 ml/min The serum creatinine calcium and phosphate phosphorus concentrations were normal Renal biopsy focal interstitial inflammation as in pyelonephritis Pyelography showed that both kidneys were of the same size but that the parenchyma of the left was reduced and all its papillae had been sloughed Similar but less marked lesions were present in the right kidney Determinations on catheter specimens showed a moderate number of W B C and R B C as well as a growth of *E coli* (70 000/ml) in the urine from the left ureter but not in that from the right With respect to concentration ability and pH of the urine as well as creatinine concentration the left kidney showed poorer function than the right (Table X)

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Catheterization of the ureters or renal veins does not involve much discomfort fo the patient, and neither measure is risky or difficult to perform for anyone with some experience Both procedures are however, somewhat lengthy particularly when combined with a functional diagnosis of the type described here Moreover they require the assistance of trained staff, special premises and — at any rate as far as renal vein catheterization is concerned — expensive equipment It is beyond the scope of the present study to attempt an assessment of the clinical value of ureteric and renal vein catheterization in general It nevertheless seems motivated to try to estimate the value — and in particular the clinical advantages — of ascertaining the presence of any asymmetry of renal function with these procedures, above all in cases in which a definite diagnosis cannot be made with simpler methods Since chronic pyelonephritis is the disease (or group of diseases) constituting the majority of the cases in the present study, the following discussion is primarily related to this complaint although other forms of renal diseases are also considered

Methods for Studies of Each Kidney Separately

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concretion had formed in the right kidney possibly in connexion with the bacteriuria At the first examination, the concentration ability of the right kidney was 9 per cent better than that of the left On the second occasion this ability was unchanged in the left kidney but had deteriorated considerably in the right (in which the concretion was present) and the difference between the two was then 3 per cent with the better value for the left kidney

For practical reasons cases of chronic pyelonephritis can be divided into two categories according to the difficulty involved in establishing the diagnosis

One category consists of cases in which the diagnosis is fairly obvious chiefly because they fulfil the cardinal criteria discussed in Chapter III The other category consists of case in which a diagnosis of chronic pyelonephritis is probable but not definite

Among the cases in the first category are those for instance in which the history indicates repeated attacks of pyelonephritis the urine contains bacteria and other elements suggestive of pyelonephritis and the roentgenologic features are characteristic *e g* deformation of calyces difference between the size of the kidneys and scarred indentations on the kidney surface

In cases in which fulfilment of the cardinal criteria makes a diagnosis of chronic pyelonephritis fairly obvious it may often seem superfluous to test for asymmetry of renal function Nevertheless it may be motivated to study the two kidneys separately to determine for instance whether bacteriuria originates from only one of them or whether function is depressed in both although a concretion has been detected in one kidney only Such a study is particularly valuable when surgery is being considered This is illustrated by the following case

CASE 97 a 37 year old woman Diagnosis chronic pyelonephritis + right sided nephrolithiasis + arterial hypertension + obesity About 2 years before the current course of treatment arterial hypertension and proteinuria had been detected in connexion with pregnancy

The 24 hour blood pressure ranged from 180/120 to 140/100 mm Hg (mean 160/110 mm) Ocular fundi changes corresponding to FH I Roentgenologic examination of the heart size and configuration normal Serum creatinine normal PAH and endogenous creatinine clearances in bladder urine 297 and 101 ml/min respectively Excretory pyelography showed kidneys of equal size (R 15 cm long L 16 cm) with large coral concretion in the right kidney Selective studies of renal function indicated that the right kidney's concentration ability and the Na concentration in its urine were substantially lower than the left kidney's 35 000 bacteria/ml were found in the right kidney's urine but none in the left PAH extraction was not tested Right sided nephrectomy was performed Although the patient's urine has been free from bacteria at several check ups her blood pressure has not fallen appreciably Histologic examination of the right kidney showed the changes associated with chronic pyelonephritis

The other category of cases — in which the diagnosis of chronic pyelonephritis is probable but not definite — consists of patients presenting symptoms and signs which are either obscured by some other renal disease or which do not satisfy the cardinal criteria Patients in the aforementioned first category need not invariably be submitted to the extensive questioning and investigations required to test these criteria In the present category on the other hand these criteria are of great value since the diagnosis can be regarded as comparatively definite if at least three of them are complied with Nevertheless useful information can be obtained by making other investigations *e g* selective studies of renal function The factor that has proved to be of paramount value for strengthening the diagnosis has

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as a result of pronounced anatomic changes, *which were established roentgenologically* (cases 35 and 39) In these cases, it was difficult to detect any functional pattern in the impairment of different renal functions This circumstance which has already been discussed in Chapter V, is in agreement with reports by *e g* Bengtsson (10b) and Kleeman *et al* (135)

The results in question nevertheless show that a bilateral study of enal function can be an aid in diagnosis, especially in a number of cases of chronic pyelonephritis in which the diagnosis was either supported by the bilateral study or would have been impossible to make without it As a rule, these patients fulfilled only a few cardinal diagnostic criteria (cases 21, 27, 33, 43 52, 91, 96)

As already mentioned, repeated determinations of renal function were made in a number of patients undergoing long term antibacterial therapy This was done in an attempt to obtain an objective measure of the value of this form of therapy In 13 of the 16 cases, the function of at least one kidney improved during therapy (cases 2 4a b, 6 7, 9 13 14, 19 20 25 82 86 90 Table X)

CASE 14 a 26 year old woman Ureteric and renal vein catheterization were performed twice (case 14 a and b) at an interval of 6 weeks and *a third time (14 c)* 13 months later On the first occasion the PAH extraction was strongly depressed on both sides particularly the left The concentration ability was also reduced by about the same amount on both sides On the third occasion considerable subjective improvement was noted In addition the endogenous creatinine clearance had more than doubled and the urine was free from bacteria Moreover both concentration ability and PAH extraction had improved on both sides The interesting feature was that — parallel to this general improvement — the difference between the extraction by the two kidneys *was much less* (7 instead of 22 per cent) On the other hand the corresponding difference with respect to concentration ability had increased the left kidney then excreting the more concentrated urine

These findings can be interpreted as bilateral improvement, in which the left kidney — particularly its concentrating apparatus — had made the better recovery

Renal function deteriorated in three patients during the interval between examinations

CASE 4 a 45 year old woman Definite improvement was observed between the first two examinations (4 a and b group A1) but later on the condition of the patient was influenced by the formation of concretions in the left renal pelvis (4 c group B1) probably owing to a recurrence of the urinary tract infection As a result the PAH extraction by the left kidney was considerably reduced but this was paralleled by an improvement in the concentration ability of both kidneys Here we have the paradoxical phenomenon of cortical function (proximal tubules) deteriorating on the affected side together with an apparent improvement in medullary function (collecting tubules and the loop of Henle)

CASE 84 a 28 year old woman She also had a recurrence of bacteriuria at the time of the second examination which may explain the reduction in PAH extraction At the first examination the PAH extraction by the two kidneys differed by 7 per cent the corresponding difference for the concentration ability being 25 per cent *The latter function was not determined on the* second occasion when the difference in PAH extraction had diminished to only 1 per cent On both occasions the patient had bilateral renal concretions and calcareous papillae although these regressed considerably during the observation period

CASE 99 a 45 year old woman also had a recurrence of the urinary tract infection (*Proteus mirabilis*) at the time of the second examination 2 years after the first In the interval a small

In the 10 cases of unilateral stenosis of the main renal artery in which the PAH extraction could be determined it was found to be on a satisfactory level with little difference between the kidneys In 3 of these cases extraction by the stenosed kidney was slightly poorer in 4 it was slightly better, whereas in 3 there was no difference between the kidneys I have been unable to trace any study in the literature in which the PAH extraction was determined bilaterally in patients with this disease

In contrast to the procedure in chronic pyelonephritis and in the other groups in this study bilateral ureteric catheterization in cases of hypertension and disease of the main renal artery was performed with the patient in a state of moderate hydration This was done to enable detection of any difference between the urinary flow from the two sides Forced hydration has been suggested as a mean of accentuating this difference e g by Hulet et al (109) Kjellbo et al (134) and Stamey et al (241) Naturally this precludes concurrent determination of the concentration ability A comparison between the osmolarity of the urine from either kidney can nevertheless be worth while in patients with unilateral stenosis of the main renal artery despite the fact that the result often varies with time if the patient is hydrated However in 2 of the 10 cases in which this determination was made the osmolarity was higher on the side with the stenosed artery lower in 2 cases and the same on both sides in six Higher osmolarity of the urine from the ischaemic kidney has been reported previously (64 65 134 220)

Howard s test (106) — which is based on White s (269) observations in experimental constriction of one of the main renal arteries in the dog (a 50 per cent reduction in urinary flow and at least a 15 per cent lower Na concentration in the urine from the ischaemic kidney) — has been an important tool in evaluation of stenosis of the main renal artery ever since its introduction in 1953 (5 34 56 57 210 241 276) In the present study the findings with respect to the urinary flow deviated from the stipulated pattern in 2 of 8 cases (in 2 of them there was only a slight difference between the kidneys) and with respect to the urinary concentration of Na in 2 of 11 cases (in 5 determinations the difference was $\leq$ 15 per cent) Table X Schlegel et al (220) however maintained that the urinary flow in the ischaemic kidney was lower but that the Na concentration in urine was the same in both kidneys in patients with hypertension which improved after nephrectomy Birchall et al (22) reported two cases in which the urinary flow was greatly reduced on the affected side In one of these patients the Na concentration in urine was *higher* for the ischaemic than for the non ischaemic kidney This particular finding was confirmed by Hulet et al (109)

Kjellbo et al (134) also reported that the Na concentration in urine from the stenosed kidney was moderately depressed compared to that from the other kidney The present series contains too few cases to permit any conclusions based on the urinary concentration of Na or creatinine

A lower RPF on the stenosed side has also been reported (65 240) This was found to apply to two of the 4 patients in the present study in whom this function could be determined

The inulin and creatinine clearances were found by Brown et al (34) to be higher on the stenosed side whereas Kjellbo et al

been demonstration of the extent of the pyelonephritic process in the tubules of each kidney — as far as this can be localized by means of the various tests available This is exemplified by the following case

CASE 27 a 21 year old woman She fulfilled 4 of the 8 diagnostic criteria tested She had a history of cystitis with and without fever as well as indefinite febrile attacks without signs of cystitis and a feeling of tension in the left flank On admission to hospital palpation over this flank elicited tenderness The left kidney was found to have poorer concentration ability than the right, and at renal vein catheterization the PAH extraction was lower on the left side These findings were compatible with the tension in the left flank and the tenderness to palpation Although compilation of the findings strongly supported the suspicions of chronic pyelonephritis the possibility of recurrent acute pyelonephritis could not be entirely ruled out

Other examples have already been given of cases in which split function tests contributed to the diagnosis of chronic pyelonephritis (cf pp 109—110)

Renal disease (except pyelonephritis) with unilateral or mainly asymmetric involvement (group B2)

In the present series most cases in this group of renal diseases showed a corresponding asymmetry in the various split function tests to that in groups A1, A2 and B1

Separate renal function tests combined with for instance roentgenologic examination have proved to be particularly valuable in patients in whom involvement by the relevant disease seems to be unilateral and operation is being considered The following case in the present series can serve as an example

CASE 107 an 18 year old woman Diagnosis dysplasia of left kidney + arterial hypertension Four years before the current investigation she had scarlet fever with proteinuria Urine analyses 2 years later showed nothing abnormal Examination in 1958 disclosed arterial hypertension (195/140 mm Hg) and she was therefore hospitalized She had no subjective complaints apart from periodic headaches

The 24-hour blood pressure ranged from 200/120 to 150/100 mm Hg (mean 179/113 mm) Ocular fundi changes corresponding to FH I Roentgenologic examination of the heart size normal ECG signs of myocardial anoxaemia Excretory pyelography showed the left kidney to be considerably smaller than the right the density of contrast medium in it was impaired and excretion delayed Renal aortography disclosed that the left main renal artery was no wider than a match although its walls showed no signs of local stenosis

In view of these findings nephrectomy was considered Bilateral renal vein and ureteric catheterization were carried out to assess the function of each kidney and hence the patient s chances of survival with only one The results clearly showed that the right kidney had largely normal function apart from a slight reduction in concentration ability (534 mOsmol/lit) The difference between the kidneys was marked with respect to clearances and PAH extraction and less pronounced with respect to concentration and acidifying ability Left sided nephrectomy was performed and histologic examination of the kidney showed malformation of the type described by Ask Upmark (3) together with slight chronic pyelitis The patient has had normal blood pressure at several subsequent controls of which the most recent was a 24 hour recording in June 1965

Arterial hypertension with stenosis or aneurysm of the main renal artery (group B3)

Arterial hypertension with involvement of the main renal artery is represented in the present study by 13 cases of unilateral stenosis of this artery one of bilateral stenosis and one case of bilateral aneurysm of the artery

term therapy in the intervals between them, and regular check ups

As stated earlier, the total renal function can in early stages of *e g* pyelonephritis show normal values this is because the function of the better kidney masks the functional defect of the affected one This circumstance can be detected only by means of bilateral studies of renal function (*cf* p 59)

In cases of renal disease in which routine examinations do not provide a definite diagnosis or in which the indications for operation are not clear demonstration of the function of each kidney separately is often of great value

(134) and Dustan *et al* (65) stated that the GFR was markedly reduced on the stenosed side In the present study the GFR — determined from the endogenous creatinine clearance — was lower on the stenosed side in 6 of the 9 cases in which it was compared and higher in 2

The bilateral study of renal function helped to clarify the indications for operation in most of my cases of hypertension and stenosis of the main renal artery The 7 cases in which the diagnosis could be verified at operation are noted in Table IV

As already pointed out, some stenosed kidneys excrete more concentrated urine and in most cases there is a retention of Na, unlike the picture in chronic pyelonephritis Cases are, of course, found with a combination of both diseases, one of which will then predominate in the functional pattern (*cf* p 101)

No typical functional pattern was displayed by the few patients in the present study who had some other renal disease than chronic obstructive and non-obstructive pyelonephritis or hypertension with stenosis of the main renal artery

Renal disease with presumed symmetric involvement of both kidneys (group B4) and cases with lower urinary tract symptoms in which renal involvement could not be ruled out (group B5)

In these patients — who were presumed to have relatively homogeneous involvement of the kidneys (group B4) or intact kidneys (group B5) — the various functions corresponded on the whole to the expectations The symmetry of renal function in cases of essential hypertension has been described by *e g* Dustan *et al* (65) and Hulet *et al* (109)

Conclusions

On the basis of the present study it can be stated that asymmetric renal function can be demonstrated in many cases of chronic pyelonephritis, as well as of such diseases which obviously affect one kidney more than the other In these cases tests for asymmetry can support the diagnosis or facilitate the choice of therapy although they are seldom necessary for adequate evaluation of the case On the other hand, a lack of asymmetry does not argue against pyelonephritis when other symptoms and signs strongly suggest the presence of this disease In diagnostically doubtful cases however, the absence of asymmetry may indicate the existence of another type of disease which involves the kidneys more homogeneously Naturally when taking into account the existence of asymmetry it does not suffice to establish whether one or another of the renal functions is asymmetric The degree of asymmetry must also be considered This is of decisive importance in certain cases *e g* if one of the kidneys has ceased to function

In cases of predominantly unilateral acute pyelonephritis a marked reduction in various functions is often found in the affected kidney if separate functional tests are made in connexion with the acute onset Normal values are frequently recorded for the other kidney If these tests are repeated some time later the functional impairment of the affected kidney will either have been restored to the normal level or will persist In the latter event one often finds not only successive deterioration of function in this kidney but also gradual signs of similar damage to the contralateral kidney This development can however — as previously illustrated (*cf* p 112) — be arrested by intensified treatment of acute attacks combined with long

term therapy in the intervals between them, and regular check ups

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In cases of renal disease in which routine examinations do not provide a definite diagnosis or in which the indications for operation are not clear, demonstration of the function of each kidney separately is often of great value

SUMMARY

Chapter I Criteria of Asymmetry

The physiologic symmetry in function of the two kidneys is discussed On the basis of the percentage difference between the functional capacity of the kidneys with respect to the individual parameters borderlines are drawn for the values regarded as indicative of asymmetric function

Chapter II Earlier Bilateral Comparisons Between Healthy Kidneys

A survey is given of investigations by other authors in which the function of the two kidneys was compared in healthy subjects

Chapter III Diagnosis of Chronic Pyelonephritis

The diagnosis of chronic pyelonephritis is discussed Eight cardinal diagnostic criteria are set up In the present series a diagnosis *of chronic pyelonephritis is made only when* at least three of these criteria are fulfilled

Chapter IV Diagnosis of Other Renal Diseases

The diagnosis of the renal diseases other *than* pyelonephritis — which are included in the present series for comparative purposes — is discussed

Chapter V Case Material

The present investigation is based on 152 patients with renal diseases consisting in 80 cases of chronic or acute non obstructive pyelonephritis (groups A1 A2) The remaining 72 patients consisted of 25 with chronic pyelonephritis and urinary tract obstruction (group B1), 11 with unilateral or mainly asymmetric renal damage (group B2) 15 with arterial hypertension and stenosis or aneurysm of the main renal artery (group B3), 16 with renal damage which could be *expected to be bilaterally homogeneous* (group B4), and 5 in whom renal involvement could not be ruled out (group B5) The comparative material comprised 11 volunteers with healthy kidneys in whom the renal extraction of para aminohippuric acid (PAH) was determined

Chapter VI Choice of Methods for Demonstrating Asymmetry

A brief survey is given of the methods available for demonstrating asymmetry of renal function In the present series bilateral renal vein catheterization and or bilateral ureteric catheterization were performed in every case *This permitted the renal extraction* of para aminohippuric acid (PAH) and the renal concentration ability to be determined in each kidney separately In several cases other split function tests were also made

Chapter VII Methods

The methods used in the present investigation are described With respect to renal vein catheterization the advantage is stressed

of using closed-circuit television for guiding the catheter — In a few cases the renal extraction of radioactive Diodrast by each kidney was studied in addition to that of PAH

Chapter VIII Sources of Error in the Clinical Physiologic Methods

The sources of error in the methods used are discussed from both the general and the physiologic aspects Particular attention is focused on the extent to which renal function may be influenced by the introduction of ureteric catheters as well as on the risk of leakage of urine outside the catheters

Chapter IX Results

The results of split function tests are given chiefly in tabular form In the subjects with healthy kidneys the extraction ability was invariably symmetric In most cases of pyelonephritis with impaired renal function the reduction was asymmetric in several parameters tested As a rule the asymmetry was greatest in patients with moderately impaired function When the kidneys were severely damaged the reduced functional capacity tended to become symmetric again In general the asymmetry of the renal concentration ability was greater than that of the extraction ability

Furthermore in chronic pyelonephritis the concentration ability was usually more reduced than the extraction ability The glomerular filtration rate was generally less reduced than the concentration ability although it was more affected than the extraction ability

In the cases of uncomplicated pyelonephritis the renal extraction ability — but not the other parameters — was on the whole significantly lower on the left side than on the right

In five cases of chronic pyelonephritis the extraction ability of each kidney was studied simultaneously at varying plasma concentrations of PAH The difference between the depression limit in the two kidneys was recorded in two of the cases

The results were less consistent in the patients with renal diseases other than chronic and acute pyelonephritis However in most cases of stenosis of the main renal artery the existence of asymmetry with respect to urinary volume osmolarity and sodium concentration was in agreement with the reports of other authors

Chapter X General Discussion

A Implications of asymmetry of individual functions The fact that in cases of pyelonephritis the PAH extraction by the left kidney was as a rule more reduced than that by the right is discussed Various explanations of this observation are suggested

B Relation between the types of functional damage and their pathophysiologic implications in the individual kidney In moderately severe pyelonephritis the renal concentration ability was generally more reduced than the extraction This was interpreted as an indication that the medulla was more damaged than the cortex In cases of acute pyelonephritis in which renal damage was slight the degree of functional impairment was about the same for both parameters The fact that this also applied in extremely severe pyelonephritis is ascribed to both the medulla and the cortex being so seriously damaged that only an inappreciable functional capacity remained in both

organs Various alternatives are discussed to explain why the medulla is more sensitive to pyelonephritic damage

C Interpretation of a decreased extraction ability Bilateral determinations were made of the upper limit of the PAH concentration in blood at which the PAH extraction no longer remained constant, but started to fall — the depression limit This permitted conclusions about whether damage to the proximal tubules was due to a regional loss of their PAH transporting cells, or to a general decrease in the capacity of these cells to transport PAH

D Clinical value of testing for asymmetry of renal function Only in relatively few cases did demonstration of asymmetry with respect to concentration and extraction ability play a decisive role in diagnosis of a renal disease However — as pointed out by other authors — demonstration of asymmetry was, in fact often a prerequisite for evaluating the indications for operation in urological cases This could be confirmed particularly as regards urinary volume and urinary sodium concentration in stenosis of the main renal artery

During the course of a renal disease, the individual renal functions could be followed in each kidney separately This was found to be of special interest in long term treatment of chronic pyelonephritis which as a rule led to improvement

AKNOWLEDGEMENTS

I am deeply indebted to Professor Bertil Josephson Head of the Clinical Central Laboratory and Docent Harje Bucht Head of the Renal Clinic for their unfailing interest invaluable advice and constructive criticism

I also take this opportunity of expressing my sincere thanks to

Docent Jonas Bergstrom, the Renal Clinic and med kand Peter Furst the Central Laboratory for stimulating discussions

Dr Erik Lundin the Departement of Roentgenology Karolinska Sjukhuset who was kind enough to evaluate all the roentgenograms

Dr Stig Ohlsson Head of the Department of Pathology who made all the histological examinations

Dr Andreas Palmlöv Department of Urology for all valuable help and for the pleasant conditions under which I was allowed to work in his department

Dr Carl Sandstrom, former head of the Department of Roentgenology who placed the resources of the department at my disposal

Professor Lars Olof Kallings and Dr Birgit Backelin the State Bacteriological Laboratory for valuable assistance and rewarding discussions

Mrs Ragna Hård Alsterborg for excellent technical and laboratory assistance

Mrs Lena Pirard for skilful and untiring secretarial help during the entire course of the investigation

Mrs Ann Marie Allgoth for drawing the figures and for secretarial assistance

Miss Paula Goldberg Miss Eva Jonsson Miss Inga Landbrink Miss Inga Markgren and Mrs Margareta Söderling Furu for valuable technical and secretarial help

Mrs Erica Odelberg, who translated the manuscript and gave generous help and intelligent advice

The expenses of the investigation were partly defrayed by grants from the Swedish Medical Research Council, the Knut and Alice Wallenberg Foundation the Forenade Liv Insurance Company and the Stockholm City Council

During part of the investigation I held a doctoral fellowship at Karolinska Institutet

Stockholm March 1966

Per Åke Örsten

organs Various alternatives are discussed to explain why *the medulla is more sensitive* to pyelonephritic damage

C Interpretation of a decreased extraction ability Bilateral determinations were made of the upper limit of the PAH concentration in blood at which the PAH extraction no longer remained constant but started to fall — the depression limit This permitted conclusions about whether damage to the proximal tubules was due to a regional loss of their PAH transporting cells, or to a general decrease in the capacity of these cells to transport PAH

D Clinical value of testing for asymmetry of renal function Only in relatively few cases did demonstration of asymmetry with respect to concentration and extraction ability play a decisive role in diagnosis of a renal disease However — as pointed out by other authors — demonstration of asymmetry was in fact, often a prerequisite for evaluating the indications for operation in urological cases This could be confirmed particularly as regards urinary volume and urinary sodium concentration in stenosis of the main renal artery

During the course of a renal disease the individual renal functions could be followed in each kidney separately This was found to be of special interest in long term treatment of chronic pyelonephritis which as a rule, led to improvement

21 BIRCHALL, R., BATSON H M., BRANNAN W Contribution of differential renal studies to the diagnosis of renal arterial hypertension with emphasis on the value of $\frac{U_{sodium}}{U_{creatinine}}$ *Amer J Med* 3, 164—170 1962

22 BIRCHALL, R., BATSON H M., MOORE, C B Hypertension due to unilateral renal arterial obstruction preliminary observations on the contribution of differential renal clearance studies *Amer Heart J* 56 616—628 1958

23 BOWMAN R L., TRANTHAM H V CAULFIELD P A An instrument and method for rapid dependable determination of freezing point depression *J Lab clin Med* 43 310—315 1954

24 BOYD J D MURDOCH, H R The radiorenogram as a measure of renal function *Arch intern Med* 109 654—659 1962

25 BRADLEY S E., CURRY J J., BRADLEY G P Renal extraction of p-aminohippurate in normal subjects and in essential hypertension and chronic diffuse glomerulonephritis *Fed Pr c* 6 79—80 1947

26 BRANNAN W., BIRCHALL, R. BATSON H M KITTREDGE W E Dehydrated hydrated excretory urography in the diagnosis of renal arterial hypertension *Trans Amer Ass gen urin Surg* 55 43—49 1963

27 BRICKER N S., DEWEY R R., LUBOWITZ, H Studies in experimental pyelonephritis simultaneous and serial investigations of a pyelonephritis and intact kidney in the same animal *Clin Res* 6 297—93 1958

28 BROD J Chronic pyelonephritis *Lancet* 270 973—981 1956

29 BROD J *Chronische pyelonephritis* VEB Verlag Volk und Gesundheit, Berlin, 130 pp 1957

30 BROD J *Die Nieren* VEB Verlag Volk und Gesundheit Berlin 1096 pp 1964

31 BROD J PRAT V., DEJDAR, R Early functional diagnosis of chronic pyelonephritis with some remarks on the pathogenesis of the pyelonephritic contracted kidney *Biology of pyelonephritis* Little Brown & Co Boston 311—330 1960

32 BROD J SIROTA J H Effects of emotional disturbance on water diuresis and renal blood flow in the rabbit *Amer J Physiol* 157 31—39 1949

33 BRODWALL, E K Renal extraction of PAH in normal individuals *Scand J clin Lab Invest* 16 1—5 1964

34 BROWN J J OWEN K PEART W S ROBERTSON J I S SUTTON D The diagnosis and treatment of renal artery stenosis *Brit Med J* 2 327—342 1960

35 BRUN C. Klorbestemmelse En mikrometode *Nord Med* 42 1774—1776 1949

36a BRUN C A rapid method for the determination of para aminohippuric acid in kidney function tests *J Lab clin Med* 37 955—958 1951

36b BRUN C Personal communication 1965

37 BRUN C. RAASCHOU F Kidney biopsies *Amer J Med* 24 676—691 1958

38 BRUN C. RAASCHOU F Recognition of pyelonephritis in percutaneous renal biopsies *Biology of pyelonephritis* Little Brown & Co Boston 225—239 1960

39 BRUN C RAASCHOU F Percutaneous renal biopsy in pyelonephritis *Ciba Symp on renal biopsy — clinical and pathological significance* J & A Churchill Ltd., London 245—261 1961

40 BUCHT H Examination of the renal plasma flow by means of para aminohippuric acid (PAH) using one intramuscular injection *Scand J clin Lab Invest* 1 126—130 1949

41 BUCHT H Studies on renal function in man — with special reference to glomerular filtration and renal plasma flow in pregnancy *Scand J clin Lab Invest* 3 1—64 1951

42 BUCHT H Långtid behandling av urinvägsinfektioner *Med Arb* 8 241—250 1965

43 BUCHT H., BERGSTRÖM J., LINDHOLMER, B., WIJNBLADH H., HÖKFELT B Catheterization of the left

REFERENCES

1 AMPLATZ K Two radiographic tests for assessment of renovascular hypertension *Radiology* 79 807—815 1962

2 ANDRIOLE V T EPSTEIN F H Prevention of pyelonephritis by water diuresis evidence for the role of medullary hypertonicity in promoting renal infection *J clin Invest* 44 73—79 1965

3 ASK UPMARK E Über juvenile maligne Nephrosklerose und ihr Verhaltnis zu Storungen in der Nierenentwicklung *Acta path microbiol scand* 6 383—442, 1929

4 BACON R D Respiration pyelography — a study of renal motion in health and disease *Amer J Roentgenol* 71 71—82 1940

5 BALDWIN, D S Function of the separate kidneys in hypertension of unilateral renal origin *Progr cardiovasc Dis* 4 134—147 1961

6 BALDWIN D S HULET W H BIGGS A W GOMBOS E A CHASIS H Renal function in separate kidneys of man II Hemodynamics and excretion of solutes and water in essential hypertension *J clin Invest* 39 395—404 1960

7 BALINT P FEKETE A FORGACS I Quantitative considerations on the storage of clearance substances in the kidney *Clin Sci* 26 345—350 1964

8 BEESON P B Factors in the pathogenesis of pyelonephritis *Yale J Biol Med* 28 81—104 1955

9 BEESON P B ROWLEY D The anti complementary effect of kidney tissue Its association with ammonia production *J exp Med* 110 685—697 1959

10a BENGTSSON U Primar kronisk pyelonefrit Ett kliniskt 5 års material *Svenska Lak Tidn* 56 497—517 1959

10b BENGTSSON U A comparative study of chronic non obstructive pyelonephritis and renal papillary necrosis *Acta med scand Suppl* 388 71 pp 1962

11 BENGTSSON U HOOD B Abuse of phenacetin containing analgetics and renal damage *Progress in pyelonephritis* F A Davis Company Philadelphia 297—306 1965

12 BERGLUND F Urinary excretion patterns for substances with simultaneous secretion and reabsorption by active transport *Acta physiol scand* 52 276—290 1961

13 BERGSTRÖM J Muscle electrolytes in man *Scand J clin Lab Invest* 14 *Suppl* 68 110 pp 1962

14 BERGSTRÖM J BUCHT H EK J JOSEPHSON B SUNDELL H WERKÖ L The renal extraction of para aminohippurate in normal persons and in patients with diseased kidneys *Scand J clin Lab Invest* 11 361—375 1959

15 BERGSTRÖM J BUCHT H EK, J JOSEPHSON B ÖRSTEN P Å The influence of the plasma concentration on the renal extraction of para aminohippurate in kidney diseases *Scand J clin Lab Invest* 11 376—387 1959

16 BERGSTRÖM J BUCHT H JOSEPHSON B Determination of the renal blood flow in man by means of radio active diodrast and renal vein catheterization *Scand J clin Lab Invest* 11 71—81 1959

17 BERGSTRÖM J HULTMAN E Normal plasma sodium values *Lancet* I 113—1133 1962

18 BERLYNE G M Distal tubular function in chronic hydronephrosis *Quart J Med* 30 339—355 1961

19 BERNSTEIN L M HAMBY W M Unilateral urine sampling utilizing external urteteral compression *New Engl J Med* 269 1093—1099 1963

20 BETTGE S ROTHAUGE C F Die Bedeutung der Clearanceuntersuchung getrennter Nierenharne fur die Operationsindikation bei chirurgischen Nierenerkrankungen *Z Urol* 50 544—564 1957

hypertension *Circulation* 23 34—41 1961

66 EDLING N P G EDVALL, C A HELANDER, C G Correlation of urography and tests of renal function *Acta radiol (Stockh)* 54 433—438 1960

67 EDLING N P G EDVALL, C A., HELANDER, C G Correlation of roentgen anatomic changes and functional tests of the kidneys in hyperparathyreodism *Acta radiol (Stockh)* 55 43—48 1961

68 EDVALL, C A Determination of unilateral renal function in urology Unilateral selective clearances and the extraction ratio of PAH (EPAH) determined by renal vein catheterization *Acta chir scand* 114 303—309 1957

69 EDVALL, C A Unilateral renal function in chronic pyelonephritis and renal papillary necrosis — A study on the function of the separate kidneys with the aid of selective renal clearance and renal vein catheterization *Acta chir scand* 115 11—17 1958

70 EDVALL, C A Renal function in hyperparathyreoidism — A clinical study of 30 cases with special reference to selective renal clearance and renal vein catheterization *Acta chir scand Suppl* 229 56 pp 1958

71 EK, J The influence of heavy hydration on the renal function in normal and hypertensive man *Scand J clin Lab Invest* 7 *Suppl to vol* 7 77 pp 1955

72 EK, J HULTMAN E A new method for determining aldosacchanides *Scand J clin Lab Invest* 9 315—316 1957

73 EPSTEIN F H BECK, D CARONE, F A., LEVITIN H MANITIUS A Changes in renal concentrating ability produced by parathyroid extract *J clin Invest* 38 1 14—1121 1959

74 EPSTEIN F H CARONE, F A., LEVITIN H MANITIUS A., BECK, D Loss of concentrating power and its relationship to pathology of the medulla in some experimental and clinical diseases of the kidney *Biology of pyelonephritis* Little Brown & Co., Boston, 295—304 1960

75 ERICSSON N O., IVEMARK, B Renal dysplasia and pyelonephritis in infants and children *Arch Path* 66 255—263 1958

76 ERIKSSON E GRANBERG P O Studies on the renal excretion of Citanest® and Xylocaine® *Acta anaesth scand Suppl* 16 79—85 1965

77 FENCL, V Renal correction of metabolic acidosis in chronic pyelonephritis *Excerpta med (Amst) Intern Congr Series* No 29 p 86 1960

78 FINNERTHY F A MASSARO G DeC., KAKAVIATOS N CHUPKOVICH V Incidence of unsuspected urinary tract infection in normal pregnant and toxemic patients *New Engl J Med* 265 534—537 1961

79 FORSSMANN W Die Sondierung des rechten Herzens *Klin Wschr* 8 2085—2087 1929

80 FREEDMAN L R., BEESON P B Experimental pyelonephritis IV Observations on infections resulting from direct inoculation of bacteria in different zones of the kidney *Yale J Biol Med* 30 406—414 1958

81 FREEDMAN L R., KAMINSKAS E., BEESON P B Experimental pyelonephritis VII Evidence on the mechanisms by which obstruction of urine flow enhances susceptibility to pyelonephritis *Yale J Biol Med* 33 65—70 1960

82 FULLERTON A Unilateral diuresis *Surg Gynec Obstet* 36 16—26 1923

83 GIDLUND A Development of apparatus and methods for roentgen studies in haemodynamics *Acta radiol (Stockh) Suppl* 130 70 pp 1956

84 GOLDRING W., CHASIS H *Hypertension and hypertensive disease* Commonwealth Fund New York 96—102 1944

85 GOODGOLD A L REUBI F Appraisal of the Sternheimer Malbin urinary sediment stain in the diagnosis of pyelonephritis *Urol int (Basel)* 4 225—242 1955

86 GÖTHGEN E W McLean's renal function test in surgical diseases of the kidney *Acta chir scand* 77 90—110 1936

adrenal vein for contrast injection and steroid analysis in a case of Conn's syndrome *Acta med scand* *176* 233—241 1964

44 BUCHT, H WEHLE B ÖRSTEN P Å Furadantin vid urinvägsinfektioner *Foredrag vid Med Riksstämman 3/12 1960 Nord Med 67* 654 1962

45 BUCHT H ÖRSTEN P Å BACKELIN B Kronisk pyelonefrit *Scola postgraduata med II Svenska Läk Tidn 57* 3651—3678 1960

46 BUMPUS H C Jr Tests of function of each kidney separately — A comparison of the value of specific gravity of urine with excretion of phenolsulphonephthalein *J Urol (Baltimore) 25* 387—394 1931

47 CARGILL W H The measurement of glomerular and tubular plasma flow in the normal and diseased human kidney *J clin Invest 28* 533—538 1949

48 CASPER L Zum 50 jährigen Jubiläum der Cystoskopie *Z Urol 22* 760—762 1928

49 CHARD T COLE P G Diagnosis of significant bacteriuria in pregnancy *Lancet II* 326—327 1963

50 CHASIS H RANGES H A GOLDRING W SMITH H The control of renal blood flow and glomerular filtration in normal man *J clin Invest 17* 683—697 1938

51 CHASIS H REDISH J Effective renal blood flow in the separate kidneys of subjects with essential hypertension *J clin Invest 20* 655—661 1941

52 CHASIS H REDISH J Function of the separate kidneys in hypertensive subjects *Arch intern Med 70* 738—748 1942

53 CHASIS H REDISH J ERDMANN A Jr Application of clearance method to determination of unilateral renal blood flow in man *J clin Invest 19* 782 1940

54 CHWALLA R Das Spätschicksal unserer Nieren und Uretersteinfälle *Z urol Chir 26* 157—197 1929

55 CIFUENTES D L ALBALA I Adaptation de la formule de la clearance uréique a la mesure de la fonction rénale séparée *J Urol med chir 59* 113—128 1953

56 CONNOR T B BERTHRONG M THOMAS W C HOWARD J E Hypertension due to unilateral renal disease — with a report on a functional test helpful in diagnosis *Bull Johns Hopk Hosp 100* 241—276 1957

57 CONNOR T THOMAS W C Jr HADDOCK, L HOWARD J E Unilateral renal disease as a cause of hypertension its detection by ureteral catheterization studies *Ann intern Med 52* 544—559 1960

58 CORCORAN A C PAGE I H Applications of diphenylamine in the determination of levulose in biogical media I Determination of inulin II Determination of levulose in small amounts of blood *J biol Chem 127* 601—608 1939

59 CORTNEY M MYLLE M LASSITER W GOTTSCHALK, C Renal tubular transport of water solute and PAH in rats loaded with isotonic saline *Amer J Physiol 209* 1199—1205 1965

60 COURNAND A RANGES H A Catheterization of the right auricle in man *Proc Soc exp Biol (N Y) 46* 462—466 1941

61 DAVIES E R SUTTON D Hypertension and multiple renal arteries *Lancet I* 341—343 1965

62 DEJDAR R Die chronische Pyelonephritis in röntgenographischer Darstellung *Fortschr Röntgenstr 90* 196—220 1959

63 DEJDAR R PRAT V Das Röntgenbild der Nieren und der Harnwege bei chronischen Pyelonephritis *Z Urol 50* 1—24 1958

64 DUSTAN H P POUTASSE E F CORCORAN A C PAGE, I H Significance of renal function tests in renal hypertension *Circulation 18* 714 1958

65 DUSTAN H P POUTASSE, E F CORCORAN A C PAGE, I H Separated renal functions in patients with renal arterial disease pyelonephritis and essential

hypertension *Circulation* 23 34—41 1961

66 EDLING N P G, EDVALL C A, HELANDER C G Correlation of urography and tests of renal function *Acta radiol (Stockh)* 54 433—438 1960

67 EDLING N P G, EDVALL C A HELANDER C G Correlation of roentgen anatomic changes and functional tests of the kidneys in hyperparathyreodism *Acta radiol (Stockh)* 55 43—48 1961

68 EDVALL C A Determination of unilateral renal function in urology Unilateral selective clearances and the extraction ratio of PAH (EPAH) determined by renal vein catheterization *Acta chir scand* 114 303—309 1957

69 EDVALL C A Unilateral renal function in chronic pyelonephritis and renal papillary necrosis — A study on the function of the separate kidneys with the aid of selective renal clearance and renal vein catheterization *Acta chir scand* 115 11—17 1958

70 EDVALL C A Renal function in hyperparathyreoidism — A clinical study of 30 cases with special reference to selective renal clearance and renal vein catheterization *Acta chir scand Suppl* 229 56 pp 1958

71 EK J The influence of heavy hydration on the renal function in normal and hypertensive man *Scand J clin Lab Invest 7 Suppl to vol* 7 77 pp 1955

72 EK J HULTMAN E A new method for determining aldosaccharides *Scand J clin Lab Invest* 9 315—316 1957

73 EPSTEIN F H BECK D CARONE F A LEVITIN H MANITIUS A Changes in renal concentrating ability produced by parathyroid extract *J clin Invest* 38 1214—1221 1959

74 EPSTEIN F H, CARONE F A, LEVITIN H MANITIUS A BECK D Loss of concentrating power and its relationship to pathology of the medulla in some experimental and clinical diseases of the kidney *Biology of pyelonephritis* Little Brown & Co, Boston, 95—304 1960

75 ERICSSON N O, IVEMARK B Renal dysplasia and pyelonephritis in infants and children *Arch Path* 66 255—263 1958

76 ERIKSSON E, GRANBERG P O Studies on the renal excretion of Citanest® and Xylocaine® *Acta anaesth scand Suppl* 16 79—85 1965

77 FENCL V Renal correction of metabolic acidosis in chronic pyelonephritis *Excerpta med (Amst) Intern Congr Series No* 29 p 86 1960

78 FINNERTHY F A, MASSARO G DeC KAKAVIATOS N, CHUPKOVICH V Incidence of unsuspected urinary tract infection in normal pregnant and toxemic patients *New Engl J Med* 265 534—537 1961

79 FORSSMANN W Die Sondierung des rechten Herzens *Klin Wschr* 8 2085—2087 1929

80 FREEDMAN L R BEESON P B Experimental pyelonephritis IV Observations on infections resulting from direct inoculation of bacteria in different zones of the kidney *Yale J Biol Med* 30 406—414 1958

81 FREEDMAN L R KAMINSKAS E, BEESON P B Experimental pyelonephritis VII Evidence on the mechanisms by which obstruction of urine flow enhances susceptibility to pyelonephritis *Yale J Biol Med* 33 65—70 1960

82 FULLERTON A Unilateral diuresis *Surg Gynec Obstet* 36 16—26 1923

83 GIDLUND A Development of apparatus and methods for roentgen studies in haemodynamics *Acta radiol (Stockh) Suppl* 130 70 pp 1956

84 GOLDRING W CHASIS H *Hypertension and hypertensive disease* Commonwealth Fund New York 96—102 1944

85 GOODGOLD A L, REUBI F Appraisal of the Sternheimer Malbin urinary sediment stain in the diagnosis of pyelonephritis *Urol int (Basel)* 4 235—242 1955

86 GÖTHGEN E W McLean's renal function test in surgical diseases of the kidney *Acta chir scand* 77 90—110 1936

87 GRABER I G SHACKMAN R Di ded renal funct on stud es in hypertens on *Br t Med J 1* 1321—1326 1956
88 GRIMLUND K Njurskador pa grund av lang arig och regelbunden konsumt on av fenacet n i st mulerande syfte *Nord Med 68* p 986 1962
89 GUZE L B BEESON P B Exper mental pyelonephr t s *I Effect of ureteral I ga t on on the course of bacter al ntect on in the k dney of the rat J exp Med 104* 803—814 1956
90 HANZAWA S Zur Refraktometr e des separ erten Harns bei ch rurg schen *N erenkrankungen Tohoku J exp Med* 20 148—167 1932—33
91 HARVALD B VALDORF HANSEN F NIELSEN A Effect on the k dney of drugs conta n ng phenacet n *Lancet I* 303—305 1960
92 HAUCH E *Om nyrernes anatomi og deres udv kl g* Gyldandalske Bokhandels for lag Kobenhavn 104 pp 1901
93 HAUGEN H N The determinat on of endogenous creat n ne n plasma and urine *Scand J cl Lab Invest 5* 48—57 1953
94 HAUGEN H N HÖEG K VOGT A BLEGEN E M B lateral renal clearance *Scand J cl Lab Inve t 12* 66—70 1960
95 HELLSTRÖM J Über den prakt schen Wert den intra enosen Ind gokarm nprobe *Acta ch r cand 68* 225—253 1931
96 HENI F D e Ammon akbest mmung be der Ureterenur ne als N erenfunkt ons probe *Kl n W schr 20* 585 58 1941
97 HEYROVSKY A Determ nat on of nul n n plasma and ur ne *Cl n cl Acta 1* 470—474 1956
98 HIATT H H THOMPSON D D Some effects of intra enously adm n stered cal cium on inorgan c phosphate metabol sm *J cl n In e t 36* 573—580 1957
99 HILGENFELDT O Das Veratmungspyelo gramm *Dtsch Z Ch r 247* 411—460 1936
100 HINKLE L E EDWARDS C J WOLF S The occurrence of d ures s n humans n stressful s tuat ons and its poss ble re lation to the d ures s of early starvat on *J cl n I vest 30* 809—817 1951
101 HIX E L Uretero-renal reflex fac l tat ng renal vasoconstr ctor responses to emo t onal stress *A er J Phys ol 192* 191—197 1958
102 HÖEG K The early forms of renal tuber culos s Bacter olog cal and cl n cal f nd ngs Results of treatment w th selec t e renal resect on and the funct on of the resected k dney Separated renal fun t on stud es n pat ents and exper mental an mals *Acta ch r sca d Suppl 30* 155 pp 1962
103 HOJA W A HEFNER J D SMITH M R Asymptomat c bacter ur a in pregnancy *Obstet and Gy ec 24* 458—462 1964
104 HOLMGÅRD Å JOSEPHSON B BUCHT H ÖRSTEN P Å A new sulfonam de comb nat on des gned for treatment ot k dney and ur nary tract n fections its blood concentrat on and renal excret on *Curr ther Re 3* 397—404 1961
105 HOOGENHUIJZE W van Funkt onelle N erend agnost k *Z Ch r 41* 37 —3 8 1914
106 HOWARD J E BERTHRONG M SLOAN R D YENDT E R Rel ef of mal gnant hypertens on by nephre tomy in four pat ents w th un lateral renal vascular d sease *Tra A Ame Phycns 66* 164—169 1953
107 HRADCOVA L SCHÜCK O Separate est mat on of renal tubular reabsorpt on of water n ch ldren *C Ped at 13* 589 593 1958
108 HULET W H BALDWIN D S BIGGS A W GOMBOS E A CHASIS H Renal funct on n the se parate k dneys of man *I Hemodynam cs and excret on of solutes and wa er in normal sub ects J cl n In e t 39* 389—394 1960
109 HULET W H RICHARDSON J R Jr HAGEDORN C W Renal hemodyna m cs tubular excret on, and ur ne con en trat ng capac ty n the separa e k dneys

of subjects with es ential hypertension *J Lab clin Med 62* 787—803 1963

110 HULTENGREN N Renal papillary necrosis — a clinical study of 103 cases *Acta chir scand Suppl 277* 84 pp 1961

111 HUSFELDT E., AALKJAER, V McLean's urea concentration test in cases of surgical kidney sufferings *Acta chir scand 73* 399—43 1934

112 HUTT M S R de WARDENER, H E Correlation between renal biopsy and other diagnostic procedures in pyelonephritis *Ciba Symp on renal biopsy — clinical and pathological significance* J & A Churchill Ltd London 262—280 1961

113 JACKSON G G., GRIEBLE H Pathogenesis of renal infection *Arch intern Med 100* 697—700 1957

114 JAENIKE J R BRAY G A Effects of acute transitory urinary obstruction in the dog *Amer J Physiol 199* 1219—1227 1960

115 JENNI M Gleichzeitiges Vorkommen von Nierentuberculose und unspezifischer Pyelonephritis *Urol int (Basel) 6* 174—186 1958

116 JOHNSTONE F R C The suprarenal veins *Amer J Surg 94* 615—620 1957

117 JOSEPHSON B *Chemistry and therapy of electrolyte disorders* Amer Lecture Ser Charles C Thomas Springfield Illinois 779 pp *1961*

118 JOSEPHSON B The clinical value of the apparent serum creatinine concentration *Scand J clin Lab Invest 15 Suppl 69* 171—179 1963

119 JOSEPHSON B BUCHT H EK, J., WERKÖ L. Renal extraction, its depression and the tubular storage of para aminohippuric acid (PAH) in the healthy and in the diseased human kidney *Scand J clin Lab Invest 4* 1—14 1952

120 JOSEPHSON B GODIN A S Om bestamning av glomerulusfiltrationen Inulin och kreatininclearance *Nord Med 18* 893—914 1943

121 JOSEPHSON B GRIEG A., KAKOSSAIOS G KALLAS J Renal tubular excretion from high plasma levels of para aminohippurate (PAH) and diodrast (D) in unanaesthetized rabbits *Acta physiol scand 30* 11—21 1953

122 JOSEPHSON B KALLAS J Iodine concentration in rabbit kidneys after diodrast injection — mechanism of renal tubular excretion. *Amer J Physiol 174* 65—71 1953

123 JOSEPHSON B., WERKÖ L., BUCHT H The renal extraction of diodrast in man *Scand J clin Lab Invest 2* 149—151 1950

124 KAITZ, A L. *Urinary concentrating ability* in pregnant women with asymptomatic bacteriuria *J clin Invest 40* 1331—1338 1961

125 KAITZ, A L., HODDER, E W Bacteriuria and pyelonephritis of pregnancy A prospective study of 616 pregnant women *New Engl J Med 265* 667—672 1961

126 KARK, R., MUEHRCKE R Biopsy of the kidney in prone position *Lancet I* 1047—1049 1954

127 KASS E H Chemotherapeutic and antibiotic drugs in the management of infections of the urinary tract *Amer J Med 18* 764—781 1955

128 KASS E H Asymptomatic infections of the urinary tract *Trans Ass Amer Phycns 69* 56—64 1956

129 KASS E H Bacteriuria and pyelonephritis of pregnancy *Trans Ass Amer Phycns 72* 257—264 1959

130 KASS E H SAVAGE, W., SANTAMARINA B A. G The significance of bacteriuria in preventive medicine *Progress in pyelonephritis* F A Davis Company Philadelphia 3—10 1965

131 KEUTEL, H J Clearance Untersuchung getrennter Nierenharne *Z Urol 47* 71—81 1954

132 KIIL, F *The function of the ureter and renal pelvis pressure recordings and radiographic studies of the normal and diseased upper urinary tract of man* W B Saunders Co., Philadelphia & London, 205 pp 1957

133 KINCAID SMITH P Bacteriuria in pregnancy *Progress in pyelonephritis* F A Davis Company Philadelphia, 11—6 1965

87 GRABER I G SHACKMAN R D \ ded renal funct on stud es n hypertens on *Brit Med J 1* 1321—1326 1956

88 GRIMLUND K Njurskador på grund av lang arig och regelbunden konsumt on av fenacet n i stimulerande syfte *Nord Med 68* p 98(1962

89 GUZE L B BEESON P B Exper mental pyelonephritis I Effect of ureteral l ga t on on the course of bacterial nfect on in the k dney of the rat *J exp Med 104* 903—814 1956

90 HANZAWA, S Zur Refraktometr e des separierten Harns bei chirurg schen N erenkrankungen *Tohoku J exp Med 20 148—162 1932—33*

91 HARVALD B VALDORF HANSEN F NIELSEN A Effect on the k dney of drugs conta n ng phenacet n *Lancet 1* 303—305 1960

92 HAUCH E *Om yrernes anatomi og deres udi kl ng* Gyldandalske Bokhandels for lag Kobenhavn *104 pp 1901*

93 HAUGEN H N The determ nat on of endogenous creat n ne in plasma and ur ne *Scand J cl n Lab Inve t 5* 48—57 1953

94 HAUGEN H N HOEG K VOGT A BLEGEN E M B lateral renal clearance *Scand J cl Lab Inve t 12 66—70 1960*

95 HELLSTROM J Über den prakt schen Wert den ntra enosen Ind gokarm nprobe *Acta ch r scand 68 225—253 1931*

96 HENI F D e Ammon akbest mmung be der Ureterenur ne als N erenfunkt ons probe *Kl n W chr 20* 585 587 1941

97 HEYROVSKY A Determ nat on of inul n in plasma and ur ne *Cl n ch m Acta 1* 470—474 1956

98 HIATT H H THOMPSON D D Some *effects of intra enously admin stered cal* c um on norgan c phosphate metabol sm *J cl n In e t 36* 573—580 1957

99 HILGENFELDT O Das Veratmungspyelo gramm *Dt ch Z Ch r 247* 411—460 1936

100 HINKLE L E EDWARDS C J WOLF S The occurrence of d ures s n humans in stressful s tuat ons and its poss ble re lat on to the d ures s of early sta at on *J cl Invest 30* 809—817 1951

101 HIX E L Uretero renal reflex fac l tat ng renal vasoconstr ctor *responses to emo* t onal stress *Amer J Phy ol 19?* 191—197 1958

102 HOEG K The early forms of renal tuber culos s Bacter olog cal and cl n cal f nd ngs Results of treatment w th selec t e renal resect on and the funct on of the resected k dney Separated renal func *t on stud es n pat ents and expe mental* an mals *A ta ch r ca d Suppl 30* 155 pp 1962

103 HOJA W A HEFNER J D SMITH M R Asymptomat c bacter ur a n pregnancy *Ob tet and Gynec 4* 458—462 1964

104 HOLMGÅRD Å JOSEPHSON B *BUCHT H ÖRSTEN P Å* A new sulfonam de comb nat on des gned for treatment of k dney and ur nary tract in fect ons its blood concen rat on and renal excret on *Cur ther Re 3* 397—404 1961

105 HOOGENHUIJZE W an Funkt onelle N erend agnost k *Z Cl r 41* 377—3 8 *1914*

106 HOWARD J E BERTHRONG M SLOAN R D YENDT E R Rel ef of mal gnant hypertens on by nephrectomy n four pat ents w th un lateral renal ascular d sease *Tran A Amer Phycn 66 164—169* 1953

107 HRADCOVA L SCHÜCK O Separa e est mat on of renal *tubular reabsorpt on* of water n ch ldren *C Ped at 13* 589 593 1958

108 HULET W H BALDWIN D S BIGGS A W GOMBOS E A CHASIS H Renal funct on n the se parate k dneys of man I Hemodynam s and excret on of solutes and wa er in *normal sub ects J cl n In e 39* 389—394 1960

109 HULET W H RICHARDSON J R Jr *HAGEDORN C W Renal hemodyna* m cs tubular excret on and ur ne con en trat ng capa ty n the separa e k dneys

159 MANITUS A LEVITIN H BECK, D EPSTEIN F H On the mechanism of impairment of renal concentrating ability in potassium deficiency *J clin Invest 39* 684—692 1960

160 MANITUS A., LEVITIN H., BECK, D., EPSTEIN F H On the mechanism of impairment of renal concentrating ability in hypercalcemia *J clin Invest 39* 693—697 1960

161 MARGOLIN E G., MERRILL, J P HARRISON J H Diagnosis of hypertension due to occlusions of the renal artery *New Engl J Med 256* 581—588 1957

162 MICHIE, A J., MICHIE, C. R Urine volume a measure of renal function *Fed Proc 10* 91—92 1951

163 MICHIE, A J MICHIE, C. R Kidney function in unilateral pyelonephritis I Clinical data II Physiologic interpretations *Amer J Med* 22 179—196 1957

164 MILES B E., PATON A., de WARDENER, H E Maximum urine concentration *Brit Med J* 2 901—905 1954

165 MILES B E de WARDENER, H E Effect of emotion on renal function in normotensive and hypertensive women *Lancet II* 539—544 1953

166 MINDER J Über einige rasch orientierende Funktionsprufungsmethoden bei chirurgischen Nierenerkrankungen II Mitteilung Die Refraktion als Funktionsmethode *Z urol Chir 26* 239—264 1929

167 MINDER, J Über einige rasch orientierende Funktionsprufungsmethoden bei chirurgischen Nierenerkrankungen III Mitteilung Die Harnsaure Ausscheidung Bemerkungen uber die Harnstoffbelastung *Z urol Chir* 28 358—[illegible]83 1929

168 MOELL, H Size of normal kidneys *Acta radiol (Stockh) 46* 640—645 1956

169 MOELL, H Kidney size and its deviation from normal in acute renal failure *Acta radiol (Stockh) Suppl* 206 74 pp 1961

170 MOLLER E., McINTOSH J F van SLYKE, D D Studies of urea excretion II Relationship between urine volume and the rate of urea excretion by normal adults *J clin Invest 6* 4[illegible]—465 19[illegible]9

171 MORRIS G C Jr., CRAWFORD E S COOLEY D A., SELZMAN H M De BAKEY M E Renovascular hypertension Experience with renal artery reconstruction in 115 patients *Amer J Cardiol 9* 141—150 1962

172 MORRIS G C Jr., DeBAKEY M E Diagnosis of renal vascular disease *Amer J Cardiol 9* 756—759 1962

173 MUELLER C B SURTSHIN A CARLIN M R., WHITE, H L Glomerular and tubular influences on sodium and water excretion *Amer J Physiol* 165 411—422 1951

174 NESBITT R E L YOUNG J E Urinary tract infections during pregnancy and the puerperium Treatment with nitrofurantoin *Obstet and Gynec 10* 89—94 1957

175 NESBITT T E Determination of function of the individual kidney *J Urol (Baltimore) 71* 407—411 1954

176 NEWMAN E KATTUS A., GENECIN A., GENEST J., CALKINS E., MURPHY J Observations on the clearance method of determining renal plasma flow with diodrast, para aminohippuric acid (PAH) and para acetyl aminohippuric acid (PACA) *Bull Johns Hopk Hosp* 84 135—168 1949

177 NORDENFELT O., RINGERTZ, N Phenacetin takers dead with renal failure 27 men and 3 women *Acta med scand* 170 385—402 1961

178 OCKERBLAD N F Practical application of the creatinine renal function test *J Urol (Baltimore) 13* 411—423 1925

179 OCKERBLAD N F Further studies in the application of the creatinine kidney function test *J Urol (Baltimore) 14* 477—488 1925

180 ÖDMAN P *Percutaneous selective angiography of the main branches of the aorta* (preliminary report) *Acta radiol (Stockh) 45* 1—14 1956

181 OFSTAD J Renal arteriovenous oxygen and hemoglobin differences in acute reduction of renal blood flow *Chr Michelsens Institutt for Videnskap og Andsfrihet* [illegible] 190 pp 1965

134 KJELLBO H VIKGREN P OMAN S HOOD B Functional pattern of renal artery stenosis *Acta med scand 172* 485—495 1962

135 KLEEMAN C R HEWITT W L GUZE, L B Pyelonephritis *Medicine (Baltimore) 39* 3—116 1960

136 KNEUCKER A W Das Elektrourogramm *Urol int (Basel) 2* 263—276 1956

137 KOCVARA S PRAT V Separated clearance I A demonstration of the inaccuracy of methods based on the calculation of the volume of bladder urine *Physiol bohemoslov 4* 283—287 1955

138 KÖHLER H Harnstoffbelastung als Nierenfunktionsprufung *Z Urol 22* 475—482 1928

139 KOLBERG A Funksjonsundersökelser ved redusert nyrevolum *Nord Med 52* 1167—1168 1954

140 KORANYI A von Physiologische und klinische Untersuchungen uber den osmotischen Druck thierischer Flussigkeiten *Z klin Med 33* 1—54 1897

141 KRAMER K THURAU K DEETJEN P Hamodynamik des Nierenmarks I Mitteilung Capillare Passagezeit Blutvolumen Durchblutung Gewebshamatokrit und O_2 Verbrauch des Nierenmarkes in situ *Pflugers Arch ges Physiol 270* 251—269 1960

142 KRONIK, P Clearancebestimmungen getrennter Nierenharne *Wien med Wschr 102* 901—905 1952

143 KUSUNOKI T INOUE H TAKAYNAGI T KOBAYASHI H A study of renal function by renal vein catheterization in the urological field *Urol int (Basel) 2* 327—349 1956

144 KUTNER R Ein Versuch den Harn zu diagnostischen Zwecken mit Methylblau zu farben *Dtsch med Wschr 18* 1086—1087 1892

145 LARSEN K Method for examination of the function of each kidney separately *Acta med scand 123* 56—65 1945

146 LeBLANC A L McCANITY W J The impact of bacteriuria in pregnancy — a survey of 1300 pregnant patients *Tex Rep Biol Med 22* 336—347 1964

147 LEISHMAN A W D Bacillus coli infection of the urinary tract *Lancet 237* 971—973 1939

148 DeLEON W GARCIA A DeJESUS P L Normal weights of visceral organs in adult Philippinos *Philipp J Sci 52* 111—127 1933

149 LEYSSAC, P LUND J Simultaneous direct and indirect inulin clearance as a separate kidney function test *Scand J clin Lab Invest 13* 100—106 1961

150 LINDAHL O JOSEPHSON B The kidney function and the renal clearances of some sulfanilamide derivatives *Acta med scand 120* 195—226 1945

151 LINDEMAN, R D van BUREN C RAISZ L G Osmolar renal concentrating ability in healthy young men and hospitalized patients without renal disase *New Engl J Med 262* 1306—1309 1960

152 LINDQVIST B Instruments for catheterization of femoral vein and artery for hemodialysis Summary in English *Nord Med 72* 1193—1194 1964

153 LINDVALL, N Renal papillary necrosis — a roentgenographic study of 155 cases *Acta radiol (Stockh) Suppl 192* 153 pp *1960*

154 LUNÖE E Unilateral kvantitativ nyrefunktionsundersogelse Om teknikken ved kvantitativ opsamling af urin ved ureterkateterisation *Hospitalstiderde 73* 684—692 1930

155 LYTTON B Urinary infection in cystoscopy *Brit Med J 2* 547—549 1961

156 McDONALD D F Individual renal clearance in diagnosis of hypertension of renal origin *J Urol (Baltimore) 86* 289—293 1961

157 MADELOFF M S SCHWARTZ, F D BORGES F J ENTWISLE G REVELL, S T R YOUNG J D Jr Differential renal clearance patterns in the evaluation of hypertension *J Urol (Baltimore) 87* 258—272 1962

158 MAGNUSSON G Kidney function studies with ^{131}I tagged sodium orthoiodohippurate *Acta med scand Suppl 378* 125 pp 1962

05 REHN E GÜNZBERG I Funkt onelle Ne end gno ik mit korpe e genen Rea g n en. *Kl n W b* 2 19— 0 923

06 RELMAN A S Some cl ni al aspects of ch onic pyeloneph t s *B ology of pyelo n ph* Little B own & Co Bo on 355—360 1960

07 RELMAN A S SCHWARTZ, W B The k dney n po a s um depletion *A J M d* 24 64—773 1958

08 RELBI F C SCHROEDER, H A Can a rular hun ng be nduced n the kid ney by asoact e drugs *J I n In e* 8 114—123 1949

09 REVELL, S T R J BORGES F J ENTWISLE, G YOUNG J D J An app a al of certa n tes s for the detect on of hypertens on of un la eral renal o g n *Ann n n Med* 53 9 0—991 1960

10 RICHARDSON J R, J HAGEDORN C W BARTLEY B J HULET W H *The funct on of he nd v dual kidneys* n hype ens on of renal ascular o g n *J Lab I n Med* 65 49—63 1965

2 RINGERTZ, N Pa olog k ana om kt fynd n u a na v d fenace am s bruk *No d M d* 7 755 196

ROCHA, H FEKETY F R Acu e n flamma on n the renal co ex and me du a fo low ng he m nal in ury *J exp M d* 119 131—138 1964

3 ROCHA, H GUZE, L B FREEDMAN L R BEESON P B Expe mental p e oneph III The nfluence of lo a ed n ury n d ffe ent parts of the k ney on sus ep b l ty to ba l ary infec on *Yale J B l M d* 30 34 —354 1958

4 ROTHAUGE C F Me hod k und Wert de Clearance Un ersu hung getrenn er Ne enharne fu d e E kennung und Be handlung de e n ei gen Pye oneph s *l e h d b G U ol* 19 — 00 195

5 ROVSING T Metode ne t l at bes emme ny nes fys olog ke funk onsevne fo ud fo ny cope a on *Ho p al d nde I* 6 7 1904

6 ROWNTREE, L G GERAGHTY J T An expe men al and l n al s udy of the funct onal a t ty of the k dneys by means of phenol ulphoneph hale n *J Pha ma ol Expe Ther* 1 5 9—661 1910

217 ROWNTREE L. G GERAGHTY J T The ph hale n test *A h n e n M d* 9 84—338 191

218 SACK, G D e Ammon akbest mmung in den Ur nen bei de Harnl ter zur Pruf ung e n e tiger S orungen der Ne enfunk t on *Kl n W h* 19 497—499 1940

219 SAHEKI M Ube d e Krea n nbestim mung des Harns und Bluts be chirurg hen Ne enkrankungen *Tohoku J exp M d* 14 146—166 19 9

2 0 SCHLEGEL J U SAVLOV E D GA BOR F Some s ud es n renal hyperten s on *J U ol (B l mo e)* 81 581—595 1959

2 1 SCHNEIDER, H Uber d e Le tu g der Saure Alkal Um hlagsprobe und uber das Versagen der Ind gocarmunp obe be de E kennung von Ne enk ankungen *D h Z Ch* 49 1 3—14 1937

2 SCHROEDER E *Kl n ke ud er o e ny elunk onen ho reph e ome ede Med at l gt hen l k pas ud kl ngen af den kompen ori ke ny ehype of og hype funk on* E na Munksgaa d, Kobenha n 64—77 1944

2 3 SCHUCK, O HRADEC, E D e Ve ande ungen der tubula en Wa e esorption n der Ne ere wah end der Kathe er erung der Harnle e *Z U ol* 51 39 —400 1958

4 SCHUCK, O HRADEC, E FABIAN F SVOBODOVA E Separa e funct onal examina on of kidneys n cl n cal p a t e *B J U ol* 3 04—21 1960

2 5 SCHWEINGRUBER, R Prob eme der h on hen Verg f ung m korrb nierten *Phena et np apa a en S hw med W h* 85 116 —1166 1955

6 SCOTT R MORRIS G C., SCOTT F B SELZMAN H M FESTE, J R The d agnos appro h to renovascular hyperten on. *J U ol (Bal mo e)* 86 31—35 196

SELDINGER, S I Ca heter repla ement of the needle n pe cutaneous arte o-

182 OLSSON O Roentgen examination of the kidney and the ureter *Encyclopedia of urology* Springer Verlag Berlin—Gottingen—Heidelberg 1—328 1962

183 ÖRSTEN P Å Long term therapy of pyelonephritis *Excerpta med (Amst) Intern Congr Series No 29* 63—64 1960

184 ÖRSTEN P Å Long term treatment of chronic pyelonephritis *Acta med scand* 172 259—267 1962

185 ÖRSTEN P Å Asymmetri vid pyelonefrit med speciell hansyn till den renala extraktionen av paraaminohippursyra *Nord Med* 67 p 753 1962

186 ÖRSTEN P Å BUCHT H HOLMGÅRD Å JOSEPHSON B Long term treatment of pyelonephritis and other urinary tract infections with a new combination of sulfanilamides *J Urol (Baltimore)* 87 220—229 1962

187 PALOCZ I Über die separierte Clearance der beiden Nieren *Acta med Acad Sci hung* 2 341—347 1951

188 PANICHI S BONECHI I Determinazione radiologica delle dimensioni del rene Valori normali *Minerva med* 49 3261—3269 1958

189 PARKMAN L Indikationer for selektiva njurfunktionsundersokningar vid kirurgiska sjukdomar *Nord Med* 73 323 1965

190 PINKERTON J H M WOOD C WILLIAMS E R CALMAN R M Sequelae of urinary infection in pregnancy A five year follow up *Brit Med J* 2 539—542 1961

191 POURTEYRON E Étude comparative sur l anatomie et la pathologie des deux reins Paris 1872 (Cit by HAUCH E *Om nyrernes anatomi og deres udvikling* Kobenhavn 104 pp 1901)

192 POUTASSE E DONNELLY A DUSTAN H P Separated kidney function tests in hypertensive patients *Surg Forum* 9 826—830 1958

193 PRAT V Separated clearance III The effect of filling the urinary bladder on kidney haemodynamics and function in man *Physiol bohemoslov* 5 401—410 1956

194 PRAT V BROD J BENESOVA R DEJDAR, R FENCL, V HORAK O CERVINKA F KRATOCHVILOVA J., PAVKOVA L Research on chronic pyelonephritis during the first ten years of the institute for cardiovascular research *Rev Czech Med* 8 113—123 1962

195 PRAT V CAPKOVA E., HORACEK V PETRZELKOVA D The examination of separate renal tubular function in clinical practice *Brit J Urol* 30 142—151 1958

196 PRAT V KOCVARA S *Separated clearance II Changes in the activity of the kidneys following catheterization of the ureters Physiol bohemoslov* 4 288—296 1955

197 PRAT V KOCVARA S Separate clearance IV Changes in renal function in unilateral ureteral catheterization with an occlusion catheter *Physiol bohemoslov* 6 539—546 1957

198 QUESNE L P le LEWIS A A G Postoperative retention of water and sodium *Ciba Symp on the Kidney* J & A Churchill Ltd London 193—208 1954

199 RAABE S Fortschritte in der functionellen Nierendiagnostik VI (Schluss) Mitteilung Titrationsanalyse und Mikropnosphat bestimmungen im Verlauf funktioneller Nierenuntersuchungen *Z Urol* 44 316—332 1951

200 RAASCHOU F *Studies of chronic pyelonephritis with special reference to the kidney function* Einar Munksgaard Copenhagen 260 pp 1948

201 RADO B DEUTSCH I Über die Carbamidbelastungsprobe bei Ureterkatheterung *Z urol Chir* 22 377—386 1927

202 RAPOPORT A Modification of the Howard test for the detection of renal artery obstruction *New Engl J Med* 263 1159—1165 1960

203 REHBERG P B The rate of filtration and reabsorption in the human kidney IX Studies on kidney function *Biochem J* 20 447—482 1926

204 REHM R A Urinary infections following cystoscopy *J Urol (Baltimore)* 88 301—302 1962

250 TODD L C, CROWELL, A J Separate kidney function comparative value of dye excretion (phenolsulphonephthalein) and salt excretion (specific gravity) as tests of differential kidney function *5th Med J (Bgham Ala) 18* 282—287 1925

251 TORRANCE H B DAVIES R P, CLARK, P Detection of renal arterial stenosis in hypertension by the differential renal uptake of radioactive rubidium *Lancet II* 633—635 1961

252 TRUETA J BARCLAY A E DANIEL P M, FRANKLIN K J, PRICHARD M M L *Studies of the renal circulation* Blackwell Scientific Publications Oxford, 187 pp 1947

253 TURCK M GOFFE B PETERSDORF R G The urethral catheter and urinary tract infection *J Urol (Baltimore) 88* 834—837 1962

254 TURCK, M, GOFFE, B PETERSDORF R G Bacteriuria of pregnancy — Relation to socioeconomic factors *New Engl J Med 266* 857—860, 1962

255 ULFENDAHL, H R Studies on the intrarenal separation of red cells and plasma *Acta Univ Upsaliensis Abstr* p 9 1962

256 ULFENDAHL, H R Personal communication 1965

257 VEJLSGAARD R Bacteriuria in patients with diabetes mellitus (a controlled study) *Progress in pyelonephritis* F A Davis Company Philadelphia 478—491 1965

258 VERNEY E B Absorption and excretion of water The antidiuretic hormone *Lancet II* 739—744 781—783 1946

259 VERTES V GRAUEL J A GOLDBLATT H Renal arteriography separate renal function studies and renal biopsy in human hypertension — Selection of patients for surgical treatment *New Engl J Med 70* 656—659 1964

260 VOELCKER, F JOSEPH E Funktionelle Nierendiagnostik ohne Ureterenkatheter *Munch med Wschr 50* 1058—1089 1903

261 VONDRA N MOLNAR J, KOVACSI L FÖLDI M Data to the glomerular filtration based on clearance examinations on separated kidneys *Acta urol hung 1* 81—96 1947

262 VUUST P Diagnosen af de kirurgiske nyrelidelser *Jydsk Med Selsk Forhandlinger* 103—117 1936

263 de WARDENER, H E (Cit by le QUESNE & LEWIS) *Ciba Symp on the Kidney* J & A Churchill Ltd London, 193—208 1954

264 de WARDENER H E *The kidney An outline of normal and abnormal structure and function* J & A Churchill Ltd London 338 pp 1958

265 WARREN J V, BRANNON E S, MERRILL, A J A method of obtaining renal venous blood in unanesthetized persons with observations on the extraction of oxygen and sodium para aminohippurate *Science 100* 108—110 1944

266 WEDEEN R P GOLDSTEIN M H, LEVITT M F The radioisotope renogram in normal subjects *Amer J Med 34* 765—774 1963

267 WEISS S, PARKER, F Relation of pyelonephritis and other urinary tract infections to arterial hypertension *New Engl J Med 223* 959—967 1940

268 WHITE H L The excretion of sodium in relation to glomerular filtration *Renal function Trans 2nd Macy conf* Josuah Macy jr Foundation New York, p 127 1950

269 WINBERG J Renal function in water losing syndrome due to lower urinary tract obstruction before and after treatment *Acta paediat (Uppsala) 48* 149—163 1959

270 WINTER, C C A clinical study of a new renal function test the radioactive diodrast renogram *J Urol (Baltimore) 76* 182—196 1956

271 WOLF G A The effect of pain on renal function *Res Publ Ass nerv ment Dis 23* 358—364 1943

272 WRONG O M Tests of renal function *Renal disease* Blackwell Scientific Publications Oxford 440—460 1962

graphy A new technique *Acta radiol (Stockh) 39* 368—376 1953

228 SEMB C KOLBERG A HOEG K KIIL F Functional tests in selective kidney surgery *Acta chir scand 109* 248—254 1955

229 SENSENBACH W Effects of unilateral nephrectomy in treatment of hypertension An evaluation *Arch intern Med 73* 123—130 1944

230 SIERRA M A GABLES C LUPAREL LO F J LEVIN J R Vertebral osteomyelitis and urinary tract infection *Arch intern Med 108* 128—131, 1961

231 VAN SLYKE D D STILLMAN E CULLEN G E Studies of acidosis XIII A method for titrating the bicarbonate content of the plasma *J biol Chem 38* 167—190 1919

232 SMITH H W *The kidney Structure and function in health and disease* Oxford University Press New York 1049 pp 1951

233 SMITH H W Unilateral nephrectomy in hypertensive disease *J Urol (Baltimore) 76* 685—701 1956

234 SMITH H W *Principles of renal physiology* Oxford University Press New York 237 pp 1956

235 SMITH H W FINKELSTEIN N ALIMINOSA L CRAWFORD B GRABER M The renal clearances of substituted hippuric acid derivatives and other aromatic acids in dog and man *J clin Invest 24* 388—404 1945

236 SMITH H W GOLDRING W CHASIS H RANGES H A BRADLEY S E The application of saturation methods to the study of glomerular and tubular function in the human kidney *J N Y Mount Sinai Hosp 10* 59—108 1943

237 SMITH W W RANGES H A Renal clearances of iopax neoiopax and skiodan in man *Amer J Physiol 123* 720—724 1938

238 SPENCER A G Clearance studies in unilateral renal disease Recent advances in renal disease *Proc Conf London Roy Coll Phycns 22—23 July 1960* Pitman Med Publ Co Ltd London 1960

239 SPÜHLER O ZOLLINGER H U Die chronische interstitielle Nephritis *Z klin Med 151* 1—50 1953

240 STAMEY T A The diagnosis of curable renal hypertension by uretheral catheterization *Postgrad Med 29* 496—504 1961

241 STAMEY T A NUDELMAN I J GOOD P H SCHWENTKER F N HENDRICKS F Functional characteristics of renovascular hypertension *Medicine (Baltimore) 40* 347—394 1961

242 STEINITZ H Die Bedeutung der Harnstoffbelastungsprobe fur die Nierenfunktionsprufung *Z klin Med 111* 4_0—453 1929

243 STERNHEIMER R MALBIN B Clinical recognition of pyelonephritis with a new stain for urinary sediments *Amer J Med 11* 312—323 1951

244 STUDER A Attempts to induce renal lesions in the experimental animal by analgetics *Progress in pyelonephritis* F A Davis Company Philadelphia 307—311 1965

245 SUTER F Die ein und beideseitigen aufretenden Nierenkrankheiten (sog chirurgische Nierenaffektionen) *Handbuch der Inneren Medizin* Berlin *8* 859—864 1951

246 SUTER F MEYER H Beitrag zur Physiologie der normalen Harnsecretion beim Menschen *Naunyn Schmiedeberg s Arch exp Path Pharmak 32* 241—265 1893

247 THURAU K Renal hemodynamics *Amer J Med 36* 698—719 1964

248 THURAU K DEETJEN P KRAMER K Hamodynamik des Nierenmarks II Mitteilung Wechselbeziehung zwischen vascularem und tubularem Gegenstromsystem bei arteriellen Drucksteigerungen Wasserdiurese und osmotischer Diurese *Pflugers Arch ges Physiol 270* 270—285 1960

_49 TODD L C Separate kidney function Further observations on the specific gravity test and improvements in technic *J Amer med Ass 92* 228—229 1929

273 WRONG O M, DAVIES H E F The excretion of acid in renal disease *Quart J Med 28* 259—314 1959

274 WULFF H B Om urografiens varde som njurfunktionsprov *Nord Med 15* 2708—2710 1942

275 YAGO K Belastungsprobe mit Harnstoff bzw Kreatinin bei chirurgischen Nierenkrankungen *Tohoku J exp Med 20* 14—33 1932—33

276 YENDT E R KERR W K WILSON D R JAWORSKI Z F The diagnosis and treatment of renal hypertension With special reference to a case of hypertension due to stenosis of both renal arteries *Amer J Med 28* 169—187 1960